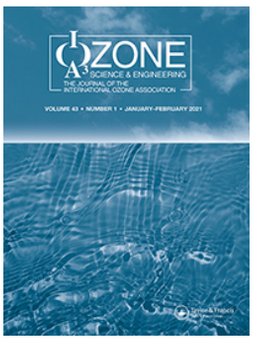

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Effectiveness of Ozone Gas on Airborne Virus Inactivation in Enclosed Spaces: A Review Study

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ABSTRACT

Due to the recent outbreak of COVID-19, the problem of protecting the individual against airborne transmission has become of great importance. This transmission occurs when virus-containing droplets enter the respiratory tract. Accordingly, the inactivation of airborne viruses plays significant role in mitigating the threat posed by a human-to-human infectious disease. The use of gas-based treatments such as ozone to decontaminate indoor air containing viruses has been increased. Ozone is a strong oxidizing agent that can be used to inactivate broad-range viruses that might be resistant to other disinfectants. The purpose of the study is to bring attention to the ozonizing of indoor air as a novel treatment for the inactivation of viruses. This review study showed that enveloped viruses (e.g., SARS-CoV-2) are more sensitive to oxidizing agents such as ozone than to non-enveloped viruses. Furthermore, some viruses such as coronaviruses have cysteine containing a sulfhydryl group that reacts with ozone gas. The study indicated that more free radicals will be formed when air humidity is higher, which could lead to higher virus inactivation. Air disinfection by ozone gas can be a promising approach for the viral deactivation of contaminated spaces in hospitals, health-care centers, dental offices, sport clubs, hotels and transportation sector, as well as all other places where viral disease outbreaks occur.

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Ozone; virus inactivation; COVID-19; disinfectant; enveloped viruses

Introduction

Viral diseases of the respiratory system are a major cause of morbidity and mortality worldwide, indicating a high economic and disease burden (Kutter et al. 2018). The relative importance of various transmission ways varies from one type of virus to another, with droplet transmission being commonly regarded as the main route for respiratory viruses (Pan, Lednicky, and Wu 2019). However, it is important to note that the relative contribution of contact, droplets, and airborne transmission depends on a combination of viral agents, host factors, and environmental factors (Otter et al. 2016). WHO stated that viral infectious diseases can be transmitted across distances relevant to indoor air by aerosols, and can result in the prevalence of the disease in a short time (Morawska and Cao 2020). Aerosols have a slow settling velocity, thus they remain suspended in the air longer and can be moved (Kutter et al. 2018). Transmission of airborne viruses from one subject to another occurs mostly in three routes: (i) direct or indirect contact with infectious secretions from infected peoples, (ii) contact of virus-containing droplets with upper respiratory tract, and (iii) inhalation of virus-containing aerosols (Pan, Lednicky, and Wu 2019). The first publications on the ability of virus to survive in the air appeared in the 1960s, where the authors reported very interesting results on the behavior of the virus in the air and the potential of transmission (Pyankov et al. 2018). A single infectious sneeze can lead to 40,000 airborne droplets, and if expelled, can move nearly 2 m to the nearest surface before falling, or it can evaporate, resulting in droplet nuclei that can remain in the air for up to 30 hours and cause lower respiratory tract infections (Coleman and Sigler 2020). Immediately after droplets are released, the liquid content starts to evaporate, and some droplets become so small that transport by airflow affects them more than gravity (Moradi, Alimohammadi, and Naderi 2016). Such small droplets are free to travel in the air and carry viruses tens of meters from where they originated (Morawska and Cao 2020). The virus-containing droplets move in the air also through convection. The convection pattern in a room can be very complicated (Anchordoqui and Chudnovsky 2020). Examination of airflow in the pediatricians' offices indicated that

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droplets were not only dispersed over the entire examination room but also accumulated in the hallway and other areas (Kutter et al. 2018). In the presence of air resistance, light particles represent Brownian motion and follow the pattern of turbulent current of the air. For droplets containing the virus, the boundary between these two behaviors depends on the size of the aerosol (Anchordoqui and Chudnovsky 2020). Sneezing and coughing lead to the production of the aerosols in the range of 1 to 500 mm. During a sneeze or a cough virus-laden droplets, typically greater than 5 mm in diameter, are expelled from the respiratory tract and directly impact susceptible individuals. A susceptible person can inhale small aerosol particles that are fine enough (<5 mm) to remain airborne for hours (Asadi et al. 2020). Virus-containing aerosols less than 2 μm in size are more likely to be infectious than the virus itself. (Tseng and Li. 2006). The persistence of such airborne viruses in space is related to a variety of factors such as temperature and relative humidity, and also virus concentration is affected by virus type, ventilation, nature of the sources and distance from the sources (Kampf 2018). The presence of viruses in aerosols showed potential airborne transmission, although many studies only quantified the content of viral RNA (Kutter et al. 2018). Significant quantities of respiratory droplets are generally expelled during speaking. A 10-min conversation with an infected person can generate 6,000 droplets (Asadi et al. 2020). Numerous viral diseases can be transmitted through the airborne route (Turgeon et al. 2017). During a military training course, an increase in the number of adenovirus infections occurred, which is associated with an increase in the detection of positive PCR air filters. Besides, correlation was observed between the disease and the rate of ventilation, with more ventilation leading to less respiratory diseases (Kutter et al. 2018). In another example, airborne transmission of viral infections containing the spread of Norwalk-like virus has been observed among school children (Morawska and Cao 2020). Measles is one of the most contagious viral diseases in humans that has been related to droplets transmission for a long period (Kutter et al. 2018). Additionally, Varicella Zoster virus (VZV) has been proven to be transmitted by the aerosol particles. The virus DNA was detected in rooms of patients without varicella in a hospital with VZV-infected patients (Bahl et al. 2020). Rhinovirus RNA was also detected in offices through air sampling (Kutter et al. 2018). Various studies focused on the detection and quantification of influenza viruses contained in droplets expelled into the air through the breathing, sneezing, and coughing of infected persons. Influenza virus RNA was detected in the air up to 3.7 m away from affected persons with the majority of viral RNA contained in droplets (Kutter et al. 2018).

Coleman and Sigler (2020) reported the first identification and quantification of airborne influenza virus in an elementary school, and the results indicate that airborne influenza A virus has the potential to spread in schools during the influenza season. Influenza virus RNA has been found in droplets collected from infected patients while they were coughing and breathing (Lindsley et al. 2016). Lindsley et al. (2016) represented that 7 of 17 patients affected with influenza emitted up to 1000 viable influenza virions over 30-min breathing. The study reported that the generation of aerosols containing viable influenza virus is common among infected people and breathing may produce more airborne infectious particles than coughing (Lindsley et al. 2016). Xie et al. (2020) also reported the presence of respiratory virus-laden particles in the exhaled breath of patients or in the air sampled at healthcare facilities.

Coronaviruses have the ability to survive in aerosol particles for long times. For instance, HCoV-229E aerosol remained infectious for 6 days (Otter et al. 2016). Otter et al. (2016) investigated the survival of MERS-CoV aerosols, and his results demonstrated a 7% reduction over 10 min. Airborne potential of MERS was surveyed by air sample analysis. Genetic materials of the virus have been detected on the inlet of air ventilation equipment and the virus was isolated from air samples (Kutter et al. 2018). The SARS prevalence was also primarily linked to healthcare centers, with 49% of the cases linked to hospitals, most probably caused by aerosols generated by patients (Kutter et al. 2018). Furthermore, a link with transmission to healthcare workers was observed when they were close to an index patient, suggesting direct contact or aerosol transmission (Kutter et al. 2018). Air samples from frequently touched surfaces in a room occupied by a SARS patient tested positive by PCR, however no virus could be cultured from these samples (Kutter et al. 2018). The SARS outbreak in Hong Kong was spread among apartments by sewer main gases drawn in by improperly sized ventilation fans and poorly maintained drains (Xia et al. 2019). In another study, air sampling from patients' rooms proves that hospitalized patients infected with SARS during the 2003 epidemic spread virus-laden droplets into the air (Kampf 2018). Currently, the emergence of a novel human coronavirus, the virus causing COVID-19, has become a global health concern leading to severe respiratory tract infections in humans (Kampf et al. 2020). Coronavirus disease 2019 (COVID-19) has been caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Ianiro et al. 2020). Human-to-human transmissions have been described

with incubation times between 2 and 10 days, facilitating its spread via droplets, contaminated hands or surfaces (Kampf, Voss, and Scheithauer 2020). Considering the many similarities between the two SARS viruses and the evidence on virus transport in general, it is highly likely that COVID-19 virus also spreads through air (Morawska and Cao 2020). Suspension of the virus in the air may be one of the several reasons that explain the sustained person-to-person spread of COVID-19 worldwide in such a short period of time (Leung, Fong, and Sun 2020). Even the largest droplets from a sneeze can spread COVID-19 virus in the air for up to 10 minutes, which allows it to reach the far end of a large room (Anchordoqui and Chudnovsky 2020). A number of studies about COVID-19 confirm aerosol transmission, and one study documented the virus at a distance of 4 meters from the infected persons, which remains viable 3 hours after release (Bahl et al. 2020). Asadi et al. (2020) reported that aerosolized COVID-19 virus remains viable in the air with a half-life of 1 h, concluding that aerosol and fomite transmission of COVID-19 virus is possible.

Air disinfection in closed spaces such as healthcare centers is an important aspect of infection prevention (Moat et al. 2009). Recently, there has been growing interest in the use of gas-based disinfectants for decontamination of indoor air (Moat et al. 2009). One of the most promising methods for virus inactivation in indoor air is ozone gas application (Tanaka et al. 2009). Ozone, the triatomic form of oxygen (O_3) is a powerful oxidizing agent, frequently used in the pharmaceutical and food industries and, water disinfection, as well as the environmental control of pathogenic microorganisms (Gholami et al. 2019; Pekovic and Kacimi 2015). Ozone is 25 times more effective than hypochloric acid (HOCl), and 2,500 to 3,000 times more potent than hypochlorite (OCl^-) (Gholami, Naderi, and Moghaddam 2018; Rojas-Valencia 2011). Ozone gas is easy and economical to generate, and safe to handle and apply (Sigstam 2014). Moreover, it is a natural compound that quickly decomposes back to oxygen with a half-life of about 20 min (Hudson, Sharma, and Vimalanathan 2009). Ozone air disinfection could be useful in various communities, hospitals (Lara-Fernández et al. 2020), healthcare facilities (Zoutman, Shannon, and Mandel 2011), dental offices (Buldur and Kapdan 2017), sport clubs (Rice and DeBrum 2011), cruise lines (Heselton et al. 2013) and other locations where outbreaks of infectious diseases are comparatively common (Pekovic and Kacimi 2015). However, ozone gas is toxic and inhalation of the gas is dangerous, its toxicity could be controlled based on its concentration and duration of exposure (Pascual, Llorca, and Canut 2007). Ozone has a number of potential advantages over other decontaminating gases and liquid chemicals and can diffuse to completely fill the space of a room (Pekovic and Kacimi 2015). Furthermore, ozone is better than other disinfectants in terms of convenience, ready dispersion after application and leaving no residues in air (Sharma and Hudson 2008). Several studies showed that ozone has 100% lethal effect on viruses (Turkmen et al. 2015). However, the initial cost of ozone generators may be high and the application over time may justify the cost (Sanchis, Pashley, and Ninham 2019). Ozone inactivation of viruses depends on the (i) ozone concentration, (ii) contact time, (iii) matrix, (iv) experimental conditions (i.e. temperature and humidity), and (v) viruses (Brié et al. 2018). Ozone has also been known as an effective treatment against airborne viruses (Turkmen et al. 2015). The standard minimum decrease in viruses that has been acted by the US EPA is a 4-log reduction in infectivity, corresponding to an inactivation of 99.99% of viruses (Sigstam 2014). Viruses, unlike "living" cells, have no mechanism of self-repair (Rowen and Robins 2020). The primary mechanism of inactivation of viruses by ozone is direct damage to the genetic material (Wu et al. 2017). Ozone may also cause damage to the outer protein capsid layer of the virus (Sanchis, Pashley, and Ninham 2019). Ozone removes up to 99% of viruses at 10 mg/L in 10 minutes, attacking mainly unsaturated fatty acids, lipid fatty acids, glycoproteins, glycolipids, amino acids, and sulfhydryl groups of some enzymes (Rojas-Valencia 2011). Moreover, ozone suppresses the virus/host cell receptor binding by altering the viral capsid proteins (Wang et al. 2018). It has been found that lipid-enveloped viruses are the most sensitive (Turkmen et al. 2015). Ozone inactivation of virus was utilized, for the first time, on mumps virus inactivation in indoor air and on internal surfaces of an experimentally contaminated room, which represents a condition closer to environmental decontamination of indoor space (Pekovic and Kacimi 2015). Recent studies represent that relatively low ozone concentration (less than 1 mg/l) and short contact time (1 min) are sufficient to inactivate 99% of viruses, such as rotaviruses, parvoviruses, and hepatitis A virus (Tseng and Li. 2006). Maier and Chu (2016) indicated the virucidal efficacy of ozone in 120 minutes. The gas at relatively high concentrations (25 ppm) has also been used to inactivate norovirus in office and hotel room spaces (Moat et al. 2009). Coronaviruses that remain infectious up to 9 days on surfaces are readily inactivated by ozone gas (Rowen et al. 2016). Various studies have reported inactivation results for different enteroviruses, adenoviruses, and several bacteriophages (Wolf 2019). High relative humidity has been applied to achieve significant

impact of the virucidal effect of ozone gas in the air. The humidification of the air can reduce the disinfection time and lead to a better efficacy (Dubuis et al. 2020). Owing to the instability of ozone molecule, it must be prepared immediately before use (Gupta and Mansi 2012). The only limitation of ozone use is the potential toxicity to humans at high concentrations. However, the health hazard can be overcome by ensuring that the spaces to be decontaminated for a short time are closed to people during disinfection in order to prevent gas leaks into the environment.

Airborne viruses

Viruses are obligatory parasites that cannot multiply outside host cells and range in size from 20 to 300 nm. They must first transfer their genomic material to the host cell that they use cellular facilities to replicate their genome and produce protein (Sigstam 2014). Viruses can be transmitted in different ways, including vector and vehicle transmission. The vehicle transmission routes can include respiratory transmission by droplets and aerosols (Tseng and Li. 2006). Outside of their host, viruses do not need any food, which makes them very stable and able to survive for a long time before infecting the new host. In addition to contact with their specific host cell, they must also be at the optimal temperature, which for most pathogenic viruses is the same as human body temperature (Sigstam 2014). Viruses can simply be represented by a genome containing either RNA or DNA, surrounded by a protein capsid and sometimes enveloped by a lipidic membrane (e.g. Influenza or COVID-19 viruses) (Sigstam 2014). Viruses are estimated to be the source of about 60% of all illnesses, from a simple cough to a serious disease such as AIDS or Ebola. They were also responsible for huge human sicknesses such as the smallpox epidemic that killed 300–500 million people in the twentieth century, or the flu epidemic in the early twentieth century responsible for more than 50 million mortalities (Sigstam 2014). Human viruses are often transmitted between persons through vertical transmission (from mother to child), through horizontal transmission (from person to person) through the exchange of body fluids (e.g. HIV, hepatitis C virus), or through respiration of excreted aerosols or droplets from infected individuals (e.g. coronaviruses, influenza virus, rhinovirus). To prevent these transmissions, the strategy is to create hurdles that cannot be penetrate by viruses or to eliminate the viruses from spaces or surfaces (Sigstam 2014). Airborne transmission of infectious viruses is further strengthened by the fact that the exhaled breath of a person with flu symptoms contains large amounts of influenza virus (Wu and Yao 2014). Currently, COVID-19 is an ongoing pandemic with almost a million confirmed cases, which has caused major disruptions to nearly all facets of everyday life around the world. The disease is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which was first identified in Wuhan, China. The virus enters human cells via the angiotensin-converting enzyme 2 (ACE2). ACE2 is a membrane protein, which is an entry point for coronaviruses found in the lungs, heart, kidneys, and intestines, and it is responsible for regulating blood pressure. According to the World health Organization (WHO), SARS-CoV-2 carries at least a 14-day incubation period (Rowen and Robins 2020). It was found that SARS-CoV-2 utilizes the ACE2 more efficiently than SARS-CoV, which may explain why the disease is very contagious (Liao et al. 2020). Large particles usually travel shorter distances. Aerosols of smaller sizes can habitually remain in the air for longer time periods and then travel over long distances. Aerosols can also settle after a prolonged time period, leading to surface contamination. A second aerosolization from the contaminated surfaces is also possible and may cause further spread of pathogens (Dubuis et al. 2020). While the exact mode of SARS-CoV-2 viral transmission is not known, a primary mode in viruses such as SARS and influenza is known to be through short-range droplets. When an infected person breathes, speaks, coughs, or sneezes micron sized droplets containing the virus are expelled into the air (Liao et al. 2020). Infections acquired in hospital settings are a major concern for patients, staffs, and visitors. They are responsible for longer hospital stays, increased costs, and even patient deaths. Norovirus, influenza and rotavirus are among the most common viruses acquired in healthcare centers. Norovirus has also been a problem in other enclosed spaces, including restaurants, schools, and kindergartens, as well as airplanes and buses. The airborne transmission route has been proven to facilitate the transmission of tuberculosis, respiratory viruses such as influenza and rhinoviruses, gastrointestinal viruses such as rotavirus, and is suspected to be involved in the transmission of other pathogens such as norovirus (Dubuis et al. 2020). Depending on the route of the pathogen, the transmission of viral diseases in indoor environments can be controlled through a variety of methods, including the use of disinfectants. Air and surface disinfection methods are currently available

in hospitals, airplanes, schools, and public places (Dubuis et al. 2020).

Air disinfection

Viruses in enclosed spaces lead to increasing need for disinfection of patient rooms and other contaminated areas. To prevent viral diseases, convenient measures are needed for each possible route of transmission. One of the routes is droplet transmission, i.e., transmission through droplets from sneezing or coughing (Andersen 2019). Contact transmission occurs through viruses that attach to the surface of the skin of the hands and fingers or various objects; however, few viruses are emitted in the air (Tanaka et al. 2009). Accordingly, it is difficult to filter them out of the air or remove them from the room using an air purifier. In addition, it has been confirmed that influenza viruses that adhere to the surface of the substance can maintain their infection even 24 hours after attachment. Effective preventive measures are therefore necessary (Tanaka et al. 2009). Efficient inactivation of airborne viruses by various methods of disinfection in indoor spaces has significant role in reducing the risk of human-to-human infectious diseases (Wu and Yao 2014). The minimum reduction in the standard for viruses created by the USEPA for water treatment is the reduction of 4-log, which is related to the deactivation of 99.99% of viruses (Wu and Yao 2014). The use of disinfectants is standard method in the inactivation of airborne pathogens in indoor air. Many hospitals use formaldehyde vaporization, peracetic acid, or chlorhexidine for this purpose. Recently, the formulation of hydrogen oxide has been supported, although the effect of these factors on reducing infection transmission in hospitals is still unclear. Such methods have disadvantages such as high cost, labor intensity, and the possibility of inhalation of disinfectant vapors by hospital staff, the formation of dirty stains on glass surfaces, and the objectionable odor of disinfectant after contamination (Sharma and Hudson 2008).

In recent years, there has been an interest in using gas-based disinfectants for the inactivation of viruses from room surfaces and spaces. Among the gas disinfectants such as ozone, chloride dioxide (ClO_2), and cold plasma, ozone has been a powerful oxidant for air disinfection (Martinelli et al. 2017). Ozone gas can be an effective alternative for virucidal purposes, as it can penetrate into all room spaces (Martinelli et al. 2017). The release of ozone can also be controlled from outside the room. Thus, as a decontamination agent, gaseous ozone has potential advantages over chlorine-releasing agents and other disinfectants (Sharma and Hudson 2008). Due to its short half-life, its toxicity and reactivity, ozone must be generated on-site, where it reacts with carbon-carbon double bonds and activated aromatic structures. Ozone reacts more slowly with fatty acids and carbohydrates, while it reacts quicker with proteins, amines, amino acids, nucleic acids, and protein functional groups (Martinelli et al. 2017). Since the gas can penetrate into all areas of the room, it is much more efficient than liquid sprays (Hudson, Sharma, and Vimalanathan 2009). The only significant drawback is its ability to corrode certain materials, such as natural rubber, long-term exposure, and its potential toxicity to humans (Hudson, Sharma, and Petric 2007). By ensuring that the room is temporarily closed to people during treatment, health risks are eliminated and sealed to prevent gas from escaping into the environment. If necessary, the sensitive material can be temporarily covered or removed (Hudson, Sharma, and Vimalanathan 2009). In addition, ozone gas can be quickly removed after treatment using a catalytic converter, which can return ozone to oxygen within minutes (Hudson, Sharma, and Petric 2007).

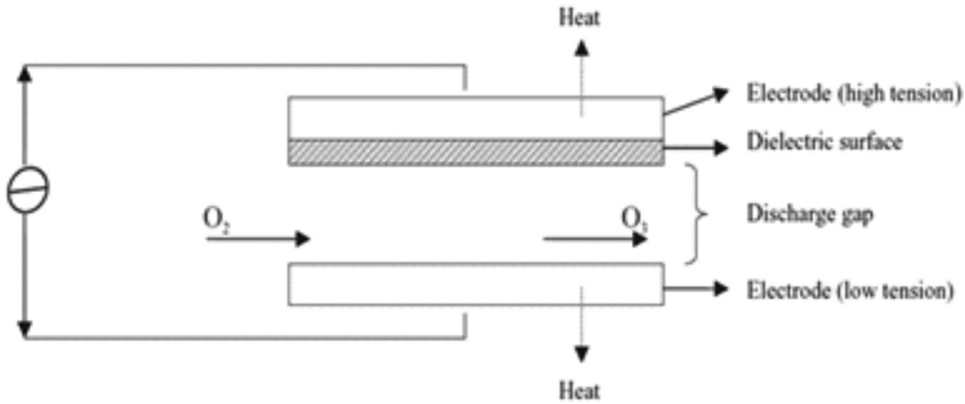

Figure 1. Schematic diagram of ozone generation by corona discharge method.

Ozone properties and generation

Ozone was initially detected by a German chemist, Christian Friedrich Schönbein, in 1839. In 1896, Nikola Tesla patented the first ozone generator in the United States, and the invention of ozone generator accelerated the widespread use of ozone, including in medical usage (Xiaoqi 2018). Ozone is triatomic oxygen (O_3) and the strongest oxidant in nature (Rowen and Robins 2020). This gas with a molecular weight of 48 g/mol is highly reactive with a lot of extra energy (□143 KJ/mol) (Zoutman, Shannon, and Mandel 2011). Ozone has a standard oxidation potential of 2.07 V *(03 + 2~++ 2e* –.H. *02+* H20) (Adachi 2001). Because of these resonance structures, ozone has the capability to react as a dipole, or as an electrophilic agent (Adachi 2001). It has a half-life of 40 min at 20 °C and of about 140 min at 0 °C (Jani et al. 2012). It has a pungent odor and is similar to "fresh air after a lightning." Ozone is a pale blue gas at room temperature that is produced from dry air but is colorless if produced with high-purity oxygen (Lindsley et al. 2016). Ozone rapidly dissolves and reacts with inorganic and organic molecules in water to create free radicals of oxygen (Xiaoqi 2018). At room temperature, ozone is an unstable gas. Ozone readily degrades but has a longer half-life in the gaseous state than in aqueous solution. Ozone is relatively stable in air but highly unstable in water, decomposes in a very short time. It cannot be stored and must be generated continuously. It is readily detectable at 0.01–0.05 ppm level (Otter et al. 2016). Ozone is a toxic gas, toxicity depends on the concentration and duration of exposure (Pascual, Llorca, and Canut 2007). At short-term exposure rates of 0.1–1.0 ppm, symptoms include headaches, nosebleeds, eye irritation, dry throat, and respiratory distress (Prabha et al. 2015). At higher exposure levels (1–100 ppm), symptoms become more severe and include asthma-like symptoms, tiredness, and decreased appetite (Gulafsha and Anuroopa 2019).

Ozone was first used in the disinfection of drinking water before being applied for medical purposes. Over the past 150 years, ozone has been broadly used to treat infections and wounds, as well as more than 100 different diseases in medicine (Xiaoqi 2018). As a bactericidal material, ozone was used during World War I to treat gaseous gangrene infections (Xiaoqi 2018). Ozone (O_3) is produced by a high energy input that divides the oxygen molecule (O_2) molecule in the air into free radical oxygen. Atomic oxygen (O) rapidly combines with existing O_2 molecules to form ozone ($3O_2 \rightarrow 2O_3$ + heat and light). Because ozone is unstable, it splits back into oxygen molecule. Ozone can be generated locally as required by several methods, three of which are currently commercially available – corona discharge, UV radiation, and electrolysis (Prabha et al. 2015). The most commercially important method is by corona discharge. In a corona discharge ozone generator, the feed gas (dried air or pure oxygen) passes between two closely spaced electrodes (one of which is coated with a dielectric material) under a nominal applied potential of ~10 kV. A silent or barrier discharge occurs when the gas becomes partially ionized, resulting in a characteristic purple glow when air is the feed gas (with high-purity oxygen the violet coloration is rarely observed) (Prabha et al. 2015). There are two electrodes in corona discharge, the high tension and low tension electrodes, which are separated by a dielectric layer in a narrow discharge gap. When electrons have enough energy to dissociate the oxygen molecule, a certain portion of these collisions occur and a molecule of ozone can be formed from each oxygen atom (Prabha et al. 2015). A schematic diagram of ozone generation using corona discharge method is shown in Figure 1. Efficiency of ozone generation by corona discharge depends on the strength of micro discharges which are influenced by a number of factors such as the gap width, gas pressure, properties of the dielectric and metal electrodes, power supply, and humidity (Prabha et al. 2015). In weak discharges, a significant portion of the energy is consumed by ions, whereas in stronger discharges, nearly all of the discharge energy is transferred to electrons responsible for the formation of ozone (Prabha et al. 2015). If air is used as the feed gas, it should be dried and be free of traces of oils and greases. Wet air produces nitrogen oxides in the ozone generator which causes erosion in the generator and requires frequent repairs and short time. If air is passed through the generator as a feed gas, 1–3% ozone is made; using high-purity oxygen may be as much as 16% ozone (Prabha et al. 2015).

Ozone transfer efficiencies vary with the number of contacting stages and are usually above 90%. However, since even a 95% ozone absorption efficiency can lead to a contactor off-gas containing a maximum 740 ppm of ozone, treatment is necessary to mitigate the ozone concentration to a standard level for discharge to the environment (Prabha et al. 2015). This can be done thermally and/or by catalytic means, and sometimes (for low concentrations) by passing through wet granular activated carbon beds (Prabha et al. 2015). Adjusting pH is not an option in air spaces, although increasing the relative humidity is an effective option. Ozone gas injection from the generator into an air space to be treated is the easiest way, and is most effective when

the air contaminants to be treated are readily reactive with ozone such as many viruses (Prabha et al. 2015).

Virus inactivation by ozone

The term disinfection is mostly used for living factors such as bacteria, but inactivation for pathogens that are not alive, such as viruses. Also, in the process of inactivation, the living or non-living factor may be inactive for a short time and become active and pathogenic again (Nardell and Nathavitharana 2020). The first ozone disinfection experiment was performed in France in 1886 (Zoutman, Shannon, and Mandel 2011). Owing to its ability to oxidize substances and destroy microorganisms, ozone has been widely used to sterilize air, purify water, and eliminate odors (Xiaoqi 2018). Various mechanisms are involved in the regulation of the effectiveness of ozone disinfection (Xiaoqi 2018). In viruses, ozone damages the viral capsid and breaks the reproductive cycle by disconnecting the contact between the virus and the cell through the peroxidation process (Xiaoqi 2018). Ozonation is a reliable approach to drastically reduce the concentration of infectious viruses (Wolf 2019). Ozone gas has various advantages as a practical antiviral agent. It can effectively penetrate any part of the room, including places that make it difficult to access common liquids and manual cleaning methods (Wolf et al. 2019). Viruses have a limited "shelf life" on surfaces. Coronaviruses have been reported to persist on surfaces up to 9 days and are rapidly inactivated by oxidizing disinfectants. It has recently been reported that gaseous ozone was able to inactivate coronaviruses (Sharma and Hudson 2008). Hudson, Sharma, and Petric (2007) have successfully tested ozone gas in laboratory and field conditions against 12 representative viruses, mainly human pathogens. He demonstrated that ozone was able to inactivate 3 logs or more different infectious viruses in rooms such as offices and hotel rooms (Hudson, Sharma, and Petric 2007). The possible mechanisms of the antiviral function include virus denaturation, lipid, and protein peroxide formation (Turkmen et al. 2015). Coronaviruses, including SARS-CoV-2 (the cause of COVID-19), are rich in cysteine. Cysteine is an amino acid carrying a sulfhydryl functional group, also called "thiol" group. Cysteine is highly vulnerable to oxidation to disulfide and changes their three-dimensional structure. Ozone oxidizes sulfhydryl functional groups immediately on contact (Viebahn-Hänsler 2003). Karlberg, Tan, and Mirazimi (2011) found cytomegalovirus loses infectivity if its thiol groups are oxidized. Re-reducing the oxidized thiols enabled the virus to regain 65% infectivity. HIV is linked to reduced sulfhydryl groups for infectivity, as is also reported for the entry of the Ebola virus into cells (Rowen et al. 2016). Like the Ebola virus, the coronavirus structure also has regions rich in cysteine, inclusive of the spike and envelope proteins (Zamora et al. 2005). Moreover, coronavirus spike protein is also rich in tryptophan, which is second to cysteine in susceptibility to oxidation (Schulz et al. 2012). Thiol groups in coronavirus and other viruses fortunately do not have self-repair mechanism, unlike "living" cells. Ozone easily acts on multi unsaturated fatty acids that occur in virus sheaths (Gupta and Mansi 2012). In the airborne phase with a short contact time, virus vulnerability to ozone can be related to the type of virus capsid structure, and with or without envelope. Complex virus capsid could provide protection from ozone inactivation, as well as the enveloped viruses which were found to have higher susceptibility to ozone (Shin and Sobsey 2003). In one study, ozone used at a concentration of 0.4–2 mg 03/L achieved a 4.2–6 log inactivation of HAV and 3.9–4.9 log inactivation of MS2. In another study, ozone at a concentration of 0.6–1.76 mg 03/L achieved a 2.96–7.00 log inactivation of MS2 and 1.63 to greater than 3.6 log inactivation of poliovirus type 3 (Adachi 2001). In order to define the interactions of ozone with viruses, candidate viruses which represent a wide range of diversity among the virus families must be selected (Adachi 2001). Tanaka et al. (2009) inactivated influenza viruses, herpes virus, adenovirus, and vesicular stomatitis virus under different ozone concentrations ranging from 800 to 1500 ppm and with this mechanism demonstrated that ozone is effective in inactivating a wide range of viruses. Tanaka et al. (2009) also conducted a study on ozone decontamination of viruses and concluded that; (1) when the ozone concentration was 0 ppm, the influenza virus that has dried on the carriers maintained a high infectivity even after 10 hours; (2) when the ozone concentration was between 10 and 20 ppm, the infectivity of the influenza virus decreased logarithmically over time; (3) when the ozone gas concentration was 20 ppm, 99.999% of the influenza virus was inactivated after 2.5 hours of fumigation, and when the ozone concentration was 10 ppm, not less than 99.99% of virus was inactivated after 3.5 hours of fumigation. There is limited data on the inactivation of viruses transmitted by the air (Tseng and Li. 2006). Ozone may first inactivate the protein capsid, and then the naked nucleic acid may be secondarily inactivated. In virus inactivation, ozone concentration, contact treatment, and the type of viral capsid protein are suggested to play important roles (Tseng and Li. 2006). Virus required ozone doses of 0.34–1.98 and 0.80–4.19 min-mg/m^3 for 90% and 99% inactivation, respectively (Tseng and Li. 2006). Compared to non-enveloped viruses, the enveloped

types are more sensitive to chemical disinfectants because they require an intact lipid envelope to infect host cells, and this envelope can be damaged by chemical and physical agents (Maier and Chu 2016).

High relative humidity is associated with superior antimicrobial effects of ozone (Zoutman, Shannon, and Mandel 2011). This humidification could reduce the treatment time and lead to a better overall efficacy (Dubuis et al. 2020). However, the optimal requirement for high humidity suggests the possible involvement of additional radicals, such as hydroxyl ion and peroxides, which could be generated under those conditions (Sharma and Hudson 2008). Tseng and Li. (2006) observed that microorganism susceptibility to ozone was significantly higher when relative humidity (RH) increased. Regarding the RH effects, the susceptibility for viruses was higher at 85% RH than that at 55% RH. This might be related to the generation of more radicals from ozone that reacted with more water vapor at higher RH (Tseng and Li. 2006). An exposure time of at least 40 minutes at 85% RH was most effective for the inactivation of the other viruses using ozone (Dubuis et al. 2020). Hudson, Sharma, and Vimalanathan (2009) also examined the role of high humidity in increasing the virus inactivation process and included this feature in the field tests. Studies have shown that the presence of ozone in high RH conditions leads to the formation of more radicals than in dry air. More free radicals can further inactivate the virus. The recommended exposure limit set by NIOSH for ozone is 0.1 ppm and this exposure level cannot be exceeded at any time (Pottinger and Marcham 2018). However, because this gas is harmful to humans at concentrations higher than this value, patients and staff should not be present during air disinfection if the concentration is higher than 0.1 ppm (Dubuis et al. 2020). Moreover, testing must be accomplished for possible ozone leakage when doors are closed in order to evaluate the feasibility of treatment. For better protection, ozone destructors can also be used and operated in the hallway near the closed door of the hospital rooms and inside them when the treatment is performed.

Conclusions

Viruses with pandemic potential including influenza and currently COVID-19 can survive for extended periods in air and may require enhanced cleaning and disinfection to assure effective infection prevention and control. Outside of their host, viruses do not need any food, which makes them very stable and able to survive for a long time before infecting a new host. Sneezing and coughing lead to the production of virus-containing particles and susceptible individuals may inhale microscopic aerosol particles. Because of increasing incidence of virus-containing aerosols, gaseous ozone can be potentially considered to be a promising method to inactivate airborne viruses. Ozone gas effectively penetrates any part of the room, including places that make it difficult to access common liquids and manual cleaning methods and also decays quickly back to oxygen with a half-life of about 20 min. As a result of this review study, we believe that the use of ozone gas has many potential applications in the viral deactivation of contaminated space. Ozone damages the viral capsid and breaks the reproductive cycle by disconnecting the contact between the virus and the cell through the peroxidation process. Our study showed that the generation of aerosols containing viable influenza virus is common among infected people and breathing may produce more airborne infectious particles than coughing. Besides, Coronaviruses such as COVID-19 virus, are rich in cysteine-containing sulfhydryl groups. Sulfhydryl groups are vulnerable to oxidation by ozone. Compared to non-enveloped viruses, enveloped types (e.g. COVID-19 virus) are more sensitive to chemical disinfectants because they require an intact lipid envelope to infect host cells, and this envelope can be damaged by chemical agents such as ozone. In addition, the study showed that high relative humidity is associated with superior antimicrobial effects of the ozone. Air humidification could reduce treatment time and lead to a better overall efficacy. However, the optimal requirement for high humidity suggests the possible involvement of additional radicals, such as hydroxyl ion and peroxides that could be generated under those conditions. Ozone inactivation of viruses depends on the (i) ozone concentration, (ii) contact time, (iii) matrix, (iv) experimental conditions (i.e., temperature and humidity), and (v) viruses. Ozone-based air disinfection can be helpful for the viral decontamination of contaminated space in hospitals, health-care institutions, dental offices, sport clubs, hotels, and transportation sector. Additionally, ozone gas toxicity can be quickly removed after treatment using a catalytic converter, which can return ozone to oxygen within minutes. In the context of the COVID-19 pandemic, future work is needed to assess the efficacy of an ozone treatment in order to reduce the transmission of this virus in hospital settings and other indoor public spaces.

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Declaration of interest statement

The authors declare that there is no conflict of interests regarding the publication of this paper.

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