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CHLORINE DIOXIDE GAS FOR THE PREVENTION OF INFECTIOUS DISEASES

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ABSTRACT

The prevention of infectious diseases caused by infectious air-floating microbes in closed and semi-closed areas is strongly needed, but it is still difficult to achieve satisfactorily because of the absence of appropriate disinfection techniques. Fumigation is one of the methods to disinfect air contaminated with infectious microbes, but it absolutely requires evacuation of people from the areas to be disinfected because the gas concentration of disinfectant employed is usually very high and toxic to animals. Currently it is demonstrated that chlorine dioxide (ClO_2) gas of extremely low concentrations that have no toxic effect to animals has strong anti-microbial activity against infectious microbes, such as bacteria and viruses. Disinfection using such low-concentration ClO_2 gas does not require evacuation of people, and could be used to disinfect room air in the simultaneous presence of people. The use of ClO_2 gas at very low concentrations may open new avenue of disinfection systems of room air without requiring evacuation of people. This review presents the details of the disinfection system of ClO_2 gas.

KEYWORDS: Chlorine dioxide, ClO_2, Gas, Bacteria, Virus, Disinfection.

INTRODUCTION

Chlorine dioxide (ClO_2) is a liquid that exhibits dark orange color below 11ºC.[1] ClO_2 starts boiling above this temperature and produces yellow gas with a characteristic odor. It is a relatively stable free radical with one unpaired electron in its molecular orbital. Its molecular structure in a liquid state was demonstrated by an X-ray diffraction analysis.[2] ClO_2 has long been used to disinfect tap water in place of chlorine in some countries. Contrary to chlorine disinfection, ClO_2 disinfection does not produce potentially carcinogenic trihalomethane.[3] Microbes disinfected by ClO_2 include bacteria, fungi, protozoa and viruses.[4] ClO_2 dissolved in water and gaseous ClO_2 are used to disinfect microbes. The detailed chemical dynamic mechanisms of inactivation of microbes by ClO_2 are reported.[5] Currently, extremely low concentrations of ClO_2 gas, on the order of 0.01- 0.05 ppm (parts per million) (volume ratio), are used to disinfect microbes in room air. The gas still possesses antimicrobial activity at these low levels.[6,7] Such extremely low levels of the gas are reported to be non-hazardous to animals,[8] and its potential use in closed and semi-closed spaces without a need of evacuation of humans is expected to open a new avenue of disinfection systems. Details of the inactivation mechanisms of microbes by ClO_2 gas are also reviewed in this paper.

CHEMICAL CHARACTERISTICS AND STRUCTURE OF CLO_2

ClO_2 is reddish-yellow gas with an unpleasant odor similar to that of chlorine at room temperature.[1] ClO_2 condenses to a reddish-brown liquid on cooling below 11ºC and freezes at -59ºC, producing red-orange crystals.[9] ClO_2 is readily soluble in water; 3 grams can be dissolved in 1 liter of water at 25ºC.[9] ClO_2 has one unpaired electron in its molecular orbital and hence is a free radical. Other chemical details of ClO_2 are presented in Table 1.[1,9] Special care is needed to handle pure ClO_2 gas at high concentrations due to its potential explosiveness and toxicity.[1,9] An explosion may result when high concentrations of the gas are exposed to strong light.

Table 1: Physicochemical characteristics of ClO_2.

Characteristic	Value
Molecular weight	67.45
Melting point	-59ºC
Boiling point	11ºC
Density of liquid	1.642 g/cm^3
Solubility in water at 25ºC	3.01 g/L
Oxidation state of chlorine	+4
Dissociation energy of first Cl-O bond	273 kJ/mol
Dissociation energy of second Cl-O bond	270 kJ/mol
Standard enthalpy of formation (ΔH_f)	102.6 kJ/mol

The structure of ClO_2 in liquid phase was observed by X-ray diffraction analysis,[2] and the gas phase structure was observed by infrared spectroscopy (Table 2).[10] Of note, the ClO_2 molecule is bent ($C_2\nu$ symmetry) with O-Cl-O angle of 116.1º.[2] The Cl-O bond length was 1.46 Å.[2] Nielsen and Woltz reported the infrared spectrum of ClO_2 gas, revealing peaks at 290, 445 (μ_2), 943.2 μ_1), 1110, 1888 ($2\mu_1$), 2040, 2215, 2473, 2967, and 3325 cm^{-1}.[10] In the solid phase, ClO_2 molecules dimerize, losing their paramagnetic behavior. The dimer $(ClO_2)_2$ becomes diamagnetic.[9] Shimakura et al. found that in the liquid phase, ClO_2 molecules do not exhibit random orientations, but present a characteristic intermolecular orientation.[2]

Table 2: Chemical structure of ClO_2.

Parameter	Value
Cl-O bond length in liquid phase	1.46 Å
Cl-O bond length in gas phase	1.491± 0.014 Å
O-Cl-O bond angle in liquid phase	116.1º
O-Cl-O bond angle in gas phase	116.5 ± 2.5º

GENERATION OF CLO_2 GAS

Numerous methods are available to generate ClO_2 gas. Given that the gas is potentially explosive at high concentrations, it is generally not transported but is generated onsite for use. The most frequently used method of ClO_2 generation involves mixing sodium chlorite ($NaClO_2$) with acids or oxidizing agents.[3] Sodium chlorite is typically employed as an aqueous solution, and acid is mixed with the solution. ClO_2 generated in the solution is bubbled by air to release it from the solution. For example, the chemical reaction involved in the use of HCl as the acid is $5NaClO_2 + 4HCl \rightarrow 4ClO_2 + 2H_2O + 5NaCl$. When ClO_2 is generated from chlorine gas (Cl_2) as a starting material, the chemical reaction is $2NaClO_2 + Cl_2 \rightarrow 2ClO_2 + 2NaCl$.[3] ClO_2 is also generated by mixing sodium chlorite with hypochlorous acid (HOCl) following the reaction of $2NaClO_2 + HOCl \rightarrow NaCl + NaOH + 2ClO_2$. In this reaction, hypochlorous acid is generated by mixing chlorine with water in the reaction of $Cl_2 + H_2O \rightarrow HOCl + HCl$.[3]

Electrochemical systems are also employed to generate ClO_2 in situ for use.[11-13] In this method, aqueous solution of sodium chlorite is placed in an electrolytic cell, where ClO_2^- becomes ClO_2 in an anode and H_2O becomes hydrogen and OH^- in a cathode.[3] The overall reaction is $2NaClO_2 + 2H_2O \rightarrow 2ClO_2 + NaOH + H_2$. ClO_2 in the aqueous solution is stripped from the solution by introducing air into the solution.[3] Bai et al. reported a sophisticated method to release ClO_2 gas in a controlled manner.[14] ClO_2 gas was generated by adhering two films together. One film is acrylate-based film loaded with sodium chlorite, and the other film is polyvinyl alcohol polymer loaded with tartaric acid. The rate of ClO_2 gas release can be controlled by tailoring film composition and its thickness. The rate of ClO_2 release is accelerated by moisture.[14] The researchers noted the usefulness of their system for food packaging. ClO_2 is also generated by exposure of a solution of sodium chlorite to ultraviolet light.[15,16] ClO_2 is generated in acidic conditions (pH 3.0-5.0), whereas hypochlorite is generated at alkaline conditions (pH 8.9-10.7).[15] Quantum yield of this photochemical reaction irradiated by 253.7 nm ultraviolet light is 0.43 to 0.94 and is maximal at pH 6.[16]

CHEMICAL REACTIONS AND FATE OF CLO_2

Whether it is an aqueous solution or gas, ClO_2 can react with numerous organic compounds. It is known to react with some free amino acids and amino acid residues in proteins.[5,17] For instance, tryptophan and tyrosine, as residues in bovine serum albumin and glucose-6-phosphate dehydrogenase of baker's yeast *Saccharomyces cerevisiae*, were oxidatively modified by an aqueous solution of ClO_2.[5] Furthermore, tryptophan becomes *N*-formylkynurenine, and tyrosine forms 3,4-dihydroxyphenylalanine (DOPA) and 2,4,5-trihyroxyphenylalanine (TOPA).[5] Oxygen atoms of ClO_2 were incorporated in the products.[5]

Napolitano et al. found that tyrosine, *N*-acetyltyrosine and DOPA react with an aqueous solution of ClO_2, consuming two molecules of ClO_2 for each reaction.[17] In the reaction of tyrosine and *N*-acetyltyrosine, phenoxyl radicals are first generated. Next, a short-lived adduct with a C-OClO bond at the 3 position of the aromatic ring is generated, ultimately forming dopaquinone and *N*-acetyldopaquinone.[17]

The mechanism of the oxidation of tryptophan is proposed as follows.[18] Two molecules of ClO_2 react with each molecule of tryptophan. The first molecule

forms a tryptophan radical, ClO_2^- and H^+. The second molecule reacts with the tryptophan radical and forms a tryptophan-OClO adduct. Finally the adduct becomes stable *N*-formylkynurenine.[18] The oxygen atoms of ClO_2 are incorporated in the product in this reaction. The amino acid cysteine also reacts with ClO_2.[19] It is proposed that the reaction involves electron transfer from cysteine anion to ClO_2 with a subsequent reaction of cysteine radical and ClO_2 to form a cysteinyl-ClO_2 adduct. The adduct finally forms pH-dependent products: cysteic acid at low pH and cystine at high pH. The tripeptide glutathione (Glu-Cys-Gly) also reacts with ClO_2.[19] ClO_2 decomposes in basic aqueous solution via three different pathways.[20] One pathway forms ClO_2^- and O_2. The other two pathways form ClO_2^- and ClO_3^-. All pathways exhibit a first-order dependence of the reaction with regard to OH^-. All the reactions are proposed to proceed by base-assisted electron-transfer mechanisms.[20]

Residues after the treatment of objects with ClO_2 are a particular concern. Basically, ClO_2 gas is rapidly broken down to chlorate (ClO_3^-) and chlorite (ClO_2^-) ions, which are further converted to chloride (Cl^-) ion.[21-26] Kaur et al demonstrated that Cl^- and ClO_3^- were formed after the treatment of cantaloupes with ^{36}Cl-labeled ClO_2 gas.[22] They treated cantaloupes with 5.1 ± 0.7 mg/L (1850 ± 254 ppm) ClO_2 gas for 10 min for fumigation. Then, they measured residues from the rind and flesh of this fruit. They detected 19.3 ± 8.0 μg of Cl^- and 4.8 ± 2.3 μg of ClO_3^- per gram of rind. They detected 8.1 ± 1.0 μg of Cl^- and no ClO_3^- per gram of flesh. Given that Cl^- is non-toxic, they concluded that fumigation of edible flesh would not pose a health concern.[22] Trinetta et al. treated vegetables and fruits with 0.5 mg/L (180 ppm) ClO_2 gas with 90 to 95% relative humidity for 10 min to disinfected pathological bacteria (*Escherichia coli*, *Listeria monocytogenes* and *Salmonella enterica*). They next rinsed the food surfaces immediately with water to remove any remaining ClO_2 and byproducts and analyzed the after-rinse water. At 24 h post treatment with ClO_2 gas, no differences in ClO_2 residues were noted between control (no ClO_2 gas treatment) and treated foods such as tomatoes and navel oranges. However, ClO_3^- was found in apples. In addition, Cl^-, ClO_2^- and ClO_3^- above control values were noted in lettuce.[23] Thus, these anions may remain on the surfaces of some agricultural foods treated with ClO_2 gas if the treatment concentration of the gas is high.

MECHANISM OF KINETICS OF ANTIMICROBIAL ACTIVITY OF CLO_2

ClO_2 has strong oxidizing activity presumably due to its free radical properties.[9] For instance, ClO_2 oxidizes tryptophan and tyrosine residues of protein and denatures proteins.[5,17,27] In this reaction, oxygen atoms of ClO_2 are incorporated in the above amino acid residues of proteins, and proteins are denatured.[5] The denaturation of proteins and inactivation of enzymes are demonstrated.[5,28,29] Finnegan et al. reported degradation (fragmentation) of bovine serum albumin and aldolase by ClO_2.[28]

Benarde et al. demonstrated that ClO_2 kills bacteria by blocking the biosynthesis of bacterial proteins.[30] Cho et al. found that ClO_2 oxidizes bacterial membrane lipids and consequently increases the permeability of the membrane.[31] On the other hand, Berg et al. reported that ClO_2 causes a loss of control of the permeability of K^+ ion and oxidative damage of the bacterial outer membrane. They concluded that *E. coli* is inactivated by these effects.[32] Roller et al. found that dehydrogenase enzymes of *E. coli* are completely inhibited by ClO_2, but this effect does not exclusively explain the inactivation mechanism of the bacteria. They suggested that inhibition of protein synthesis might have a contributory lethal effect on the bacteria.[29]

Many microorganisms are inactivated by ClO_2. Bacteria,[33-54] fungi,[55-58] viruses[59-68] and protozoa[56-58] are inactivated. ClO_2 dissolved in water has long been used to disinfect tap water.[3] The antimicrobial activities of ClO_2 are elicited as a gas. For instance, *Bacillus subtilis*, *S. enterica*, *B. anthracis*, *Francisella tularensis*, *Yersinia pestis*, *E. coli* O157:H7, and *Staphylococcus aureus* are inactivated by ClO_2 gas.[4,7,36,37,39,44-46,50,54,69] Bhagat et al. demonstrated that *S. enterica* inoculated on navel orange surfaces were inactivated to a level of 3.5 $\log_{10}$ reduction of viability with 0.1 mg/L (36 ppm) ClO_2 gas for 12 min at 22ºC with 90-95 % relative humidity.[50] A mixture of *S. enterica*, *E. coli* and *L. monocytogenes* spot-inoculated on the surface of tomatoes, cantaloupes and strawberries were treated with 10 mg/L ClO_2 (3600 ppm) gas for 180 s, and a 3-5 $\log_{10}$ reduction of viability was reported.[35]

The inactivation activity of the ClO_2 gas is elicited against the bacteria not only in their floating state in the air, but also in their attachment state on solid objects.[69] Li et al. found that spore-forming bacteria, *B. subtilis* var. *niger* attached to pieces of metal, plastic, and glass were inactivated by 800 ppm (2.2 mg/L) ClO_2 gas for 3 h to levels of 1.8 to 6.64 $\log_{10}$ reduction.[69] The results clearly indicated sporicidal activity of the gas. Interestingly, the inactivation activity is dependent on pre-humidification treatment of the test pieces.[69] Viruses are also inactivated by the gas. For instance, influenza virus, feline calicivirus, human herpesvirus, and canine distemper virus are inactivated by ClO_2 gas.[6,27,64,70,71]

Wang et al. extensively studied the kinetics of the inactivation of *B. subtilis* spores and *S. albus* inoculated on a piece of filter paper by ClO_2 gas.[46] They fitted the rate of the kill of the bacteria using a first-order kinetic model using a function of $\log_{10}(N/N_0) = -kt$, where N_0 is the initial number of cells, N is the number of surviving bacteria after time t (min) of ClO_2 gas exposure, and k (min^{-1}) is the rate constant. In the case of *B. subtilis* spores, the rate constant k is 0.09 ± 0.01 min^{-1} (n = 6) at

1 mg/L gas concentration with 70% relative humidity at 22 to 24ºC, and this value increases to 0.21 ± 0.02 min^{-1} at 5 mg/L.[46] In the case of *S. albus*, *k* was 0.15 ± 0.01 min^{-1} at 2 mg/L gas with 70% relative humidity and 0.32 ± 0.02 mg/L at 5 mg/L gas.[46] Interestingly, the rate constant *k* decreases to 0.04 ± 0.01 min^{-1} at 2 mg/L gas with 30% relative humidity, whereas it increases to 0.66 ± 0.04 min^{-1} with 90% relative humidity. Thus, the rate of killing increases along with the increase in relative humidity. The same trend is also noted in the case of *B. subtilis* spores.[46] The augmentation of the inactivation activity of ClO_2 gas upon the increase in relative humidity was also reported regarding *S. enteritidis* inoculated on eggshells.[45] Of note, the same trend of the effect of humidity was also reported regarding the inactivation of feline calicivirus. Morino et al. found that feline calicivirus placed on a glass surface and treated with 0.26 ppm (0.72 □g/L) ClO_2 gas for 24 h at 20ºC was inactivated to 1.0 log_{10} reduction (n = 4) with 45 to 55% relative humidity, whereas it was inactivated to 6.3 log_{10} reduction with 75 to 85% relative humidity.[70]

CLO_2 GAS AS A FUMIGANT

Previously high-concentration ClO_2 gas was frequently used as a fumigant to inactivate various microbes. For instance, Park and Kang demonstrated that *E. coli*, *S. typhimurium* and *L. monocytogenes* inoculated on spinach leaves and tomato surfaces were inactivated by 5 or 10 ppm (28 □g/L) ClO_2 gas.[33] *S. enterica*, *E. coli* O157:H7 and *L. monocytogenes* spotted on the surface of crops (tomatoes, cantaloupes and strawberries) were treated with 10 mg/L (3600 ppm) gas of ClO_2 for 180 s.[35] In this experiment, a 5-log_{10} reduction in colony forming unit (CFU) was noted in *S. enterica* in all crops. In contrast, a 3-log_{10} reduction in CFU was noted in *E. coli* and *L. monocytogenes*, indicating that the latter two are more resistant to ClO_2 gas.[35] A 3-log_{10} CFU reduction of *S. enterica* was also reported for mung bean sprouts at 0.5 mg/L (180 ppm) ClO_2 gas for 15 min.[37] Of note, the inactivation activity of ClO_2 gas was also found on bacterial spores.[34,36,38,42,46,47,72,73] Jenk and Woodworth reported that spore-forming bacteria *B. subtilis* planted in the artificial organs were reproducibly sterilized with 30-min dwell time with 30 mg/L ClO_2 gas (10900 ppm) with 80 to 85% relative humidity at 30ºC.[72] The D value (time required for 90% spore inactivation) was 4.4 min.[72] Lowe et al. found that 362 – 695 ppm ClO_2 gas maintained at exposures of 756 ppm-hours with 65% relative humidity achieved inactivation of *B. anthracis* and *Mycobacterium smegmatis*. The reduction of viability was greater than 6 log_{10}.[34]

TOXICITY STUDY OF CLO_2 GAS

High-concentration ClO_2 gas is toxic against numerous animals including human. Paulet and Desbrousses performed a toxicological study. Rats exposed to 10 ppm (28 μg/L) ClO_2 gas for 2 h/day for the 30-day period exhibited nasal discharge, red eyes, localized bronchopneumonia with desquamation of the alveolar epithelium and an increase in leukocytes.[74] The same group also performed other experiment using rats. They exposed rats to 1 ppm (2.8 μg/L) ClO_2 gas for 5 h/day for 5 days/week during a 2-month period. They found vascular congestion and peribronchiolar edema in the lungs of the rats.[75] However, Ogata et al[8] could not reproduce their pathological findings in rats exposed to the experimental conditions exactly as described in the paper of Paulet and Desbrousses.[75] They concluded that the most likely reason for this discrepancy might involve the fine controls of gas concentrations.[8] As noted by Ogata et al.,[8] fine controls of ClO_2 gas concentrations at such low levels might have been quite difficult to achieve at the time of Paulet and Desbrousses[75] as a gas generator with a sophisticated control system was not available.[8]

Akamatsu et al. demonstrated the rats exposed to 0.1 ppm ClO_2 gas for 24 h/day and 7 days/week for a period of 6 months were completely healthy at the end of the experiment.[76] Dalhamn conducted ClO_2 gas inhalation study on rats. Exposure to 260 ppm (720 μg/L) ClO_2 gas for 2 h resulted in ocular discharge, epistaxis, pulmonary edema, circulatory engorgement and death.[77] In contrast, exposure to 0.1 ppm (0.28 □g/L) ClO_2 gas for 5 h/day during a 10-week period did not cause any pathological effect, and he concluded that this level is NOAEL (no-observed-adverse-effect level).[77] Exposure of rats to 10 ppm (28 □g/L) ClO_2 gas for 4 h/day during a 2-week period caused respiratory tract irritation, and he concluded that this level is LOAEL (lowest-observed-adverse-effect level).[77]

GOVERNMENTAL REGULATIONS OF CLO_2 GAS CONCENTRATIONS

Given that high-concentration ClO_2 gas and liquid are explosive and toxic to animals as mentioned above, several governmental regulations have been implemented in some countries. American OSHA (Occupational Safety and Health Administration) states that the 8-hour time-weighted average of permissible exposure level of the ClO_2 gas is 0.1 ppm (0.28 μg/L).[78] The American Conference of Governmental Industry Hygienist (ACGIH) also states 0.1 ppm as a permissible level for workers working 40 hours per week and 8 hours a day.[79] NIOSH (National Institute for Occupational Safety and Health) of USA states that the permissible average 10-hour exposure level is 0.1 ppm to humans.[80] Taken together, exposure to less than 0.1 ppm ClO_2 gas appears to be safe for humans. Thus, it would be concluded that long-term exposure to ClO_2 gas at or below 0.1 ppm would be allowable to humans.

ANTIMICROBIAL ACTIVITY OF CLO_2 GAS AT EXTREMELY LOW CONCENTRAIONS

Ogata and Shibata first reported the effect of extremely low-concentration ClO_2 gas at a level of 0.03 ppm (0.084 μg/L) against influenza virus in an animal experiment[6] using a sophisticated machine to generate and deliver ClO_2 gas at finely controlled concentrations.[7] The gas

concentration was precisely controlled and accurately monitored during the study as demonstrated by recently published paper.[81] They found that the lethal activity of influenza A virus aerosol exposed to mice was dramatically reduced when 0.03 ppm ClO_2 gas was present simultaneously with the virus aerosol. All the virus-challenged mice were alive and appeared quite healthy during and after the exposure of the virus when ClO_2 gas was concomitantly present.[6] This result suggests the potential usefulness of the gas to protect human diseases caused by floating microbes in a room. A crucial point of this result is that evacuation of people from the room would not be required during the exposure to the gas because the concentration of the ClO_2 gas employed is extremely low, i.e., below the permissible exposure concentration to human as mentioned above.[79,80,82] Thus, the exposure is *not fumigation*. Currently there is no useful and reliable measure to protect humans from infection by floating microbes without requiring evacuation in closed or semi-closed spaces, such as an airplane cabin or a spacecraft. The prevention of airborne microbe infection by the extremely low-concentration of ClO_2 gas will open new avenues in the field of public health, e.g., prevention of highly pathogenic and transmissible H5N1 influenza virus.[83] The use of 0.03 ppm ClO_2 gas is also useful in prevention of mosquito-related infective diseases, such as malaria and dengue fever, given that this concentration of ClO_2 gas has a repellent effect against mosquitoes.[84]

CONCLUSION

Exposure to extremely low concentrations of ClO_2 gas, i.e., at or below 0.1 ppm (0.28 □g/L), has no harmful effect on animals, whereas 0.03 to 0.1 ppm still has inactivation activities against bacteria and virus. Such concentrations of ClO_2 gas could be used without requiring evacuation of people to prevent infections by microbes floating in air in closed or semi-closed spaces, such as in the cabins of aircrafts, living rooms and spacecraft. This effect of ClO_2 gas can be used to prevent the spread of infectious diseases, such as highly pathogenic H5N1 influenza virus, by increasing the quality of indoor air. Currently, such a disinfectant is not commercially available. To the best of our knowledge, the extremely low concentrations of ClO_2 are the only measure to prevent the infection by airborne microbes in the presence of humans.

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Declaration of Interest

The author is an employee of Taiko Pharmaceutical Co., Ltd.

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