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Ozone: A Potential Oxidant for COVID-19 Virus (SARS-CoV-2)

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ABSTRACT

Currently, no medicine has demonstrated efficacy in treating the ongoing pandemic COVID-19 caused by SARS-CoV-2 virus. Being a potent oxidant, ozone is lethal against most bacteria and viruses found in water, or on surfaces and aerosols. Ozone has also been successfully used to treat several viral diseases such as Ebola and HIV Hepatitis B and C. Using molecular modeling, this study evaluated the reactivity of ozone toward representative key molecules in the structure of SARS-CoV -2. The results show that ozone is able to attack the proteins and lipids of the virus's spikes and envelope, particularly the amino acids tryptophan, methionine and cysteine, and the fatty acids, arachidonic acid, linoleic acid, and oleic acid. Ozone also attacks the N-glycopeptides of the spike protein subunits 1 and 2, though at lower reactivity. Disruption of the structure of SARS-CoV-2 could inactivate the virus, suggesting that ozone could be an effective oxidant against COVID-19 virus. If incorrectly applied, ozone is toxic and contact with the respiratory tract must be avoided.

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Ozone; SARS-CoV-2; COVID-19; coronavirus; severe acute respiratory syndrome

Introduction

The highly infectious 2019 novel coronavirus disease (COVID-19) is caused by a new coronavirus (SARS-CoV-2) belonging to the family of severe acute respiratory syndrome (SARS) viruses. This virus has high transmission rate and has so far infected all countries in the world. SARS-CoV-2 affects severely the respiratory system of infected people and can lead, according to the World Health Organization's updated daily data, to about 7% death in infected cases (as of 15 May 2020). People who suffer from underlining health conditions are most vulnerable. The virus spreads through direct inhalation of infected droplets, or through contact with contaminated surfaces and objects (Chia et al. 2020; Zhen-Dong et al. 2020). The fecal-oral transmission route has also been postulated (Xu et al. 2020b). The virus can remain viable and infectious in aerosols for hours and on surfaces for up to days (van Doremalen et al. 2020). However, recent evidence suggests that the risk of transmission through surface contamination is lower than through the airborne route (Anderson et al. 2020; Centers for Disease Control and Prevention 2020; Morawska and Cao 2020; Setti et al. 2020). Currently, there is no vaccine nor a specific treatment for the disease, though research is at speed for developing, testing, mass-producing, and distributing specific medication and vaccine for COVID-19. People have to social engineer themselves through social-distancing and stay-at-home measures to avoid exposure to the virus. Standard hygiene measures including hand washing and mouth covering when coughing and sneezing are the first defense against SARS-COV-2 as recommended by the World Health Organization and governments across the world.

Given its strong oxidizing power, ozone is suggested here as an oxidizing agent that can potentially inactivate and destroy SARS-CoV-2. This is justified by not only the fact that ozone is a strong oxidizing agent but also by the fact that SARS-CoV-2 is an enveloped virus, which is particularly vulnerable to oxidation attack. Ozone can attack viruses at various points of their structure causing damage to the virus's integrity while making them enable to reproduce through oxidizing the viral capsid and the genetic material. Although any of the virus's structure could potentially be attacked by ozone, the structure that has most of the double bonds or groups with high electronic density will be most vulnerable to ozone oxidation. This study provides state of knowledge on ozone inactivation of viruses while emphasizing the potential of ozone as a strong oxidant to inactivate and destroy SARS-COV-2.

Method

The search engines PubMed, Web of Science, Google Scholar were searched irrespective of language and time

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using combinations of the keywords: ozone; medical ozone; COVID-19; SARS-CoV-2; coronavirus; SARS; severe acute respiratory syndrome; MERS; middle east respiratory syndrome; virus inactivation; antimicrobial. In addition, a volume of original dated published references from the International Ozone Association and handbooks was consulted. The semi-empirical PM6 method was used in Gaussian 16 to carry out a rapid molecular optimization and frequency calculations, thereby obtaining the electronic structure of molecules, providing ozone reactivity toward molecules identified in the structure of SARS-CoV-2.

Results and discussion

Ozone: a versatile virucidal

Ozone is an excellent oxidizing agent and due to its ability to kill almost all kinds of bacteria and viruses, it has been extensively used to disinfect water, wastewater, air, and food, and has gained applications in the energy, laundry, and health sectors (Khadre, Yousef, and Kim 2001; Langlais, Reckhow, and Brink 1991; Rice 2002; Rice et al. 2009; Tizaoui 2017; Tseng and Li 2008; Zanardi et al. 2016). Early research on ozone has mainly focused on inactivating waterborne viruses and have demonstrated that relatively low ozone concentrations (~1 mg/L) and short contact times (~1 min) (i.e. CT~1 mg.min/L) were sufficient to inactivate 99% of viruses, such as rotaviruses, parvoviruses, feline calicivirus, and hepatitis A virus (Akey and Walton 1985; Hirai et al. 2019; Tseng and Li 2008). A more recent viral inactivation kinetic data show that ozone is highly effective against enteric viruses with second-order ozone inactivation rate constants ranging from 4.5×10^5 to 3.3×10^6 $M^{-1}.s^{-1}$ (Wolf, von Gunten, and Kohn 2018). Evidence has also shown that ozone is effective against airborne and on-surface viruses (Hirneisen and Kniel 2013; Hudson, Sharma, and Vimalanathan 2009; Tseng and Li 2008), though the kinetics are slightly lower than in aqueous solutions (Bialoszewski et al. 2011). However, with appropriate control of ozone dose and air humidity (relative humidity: RH~80%), the virus inactivation rates increase substantially making ozone an attractive and rapid method for inactivating airborne and on-surface viruses (Hirneisen and Kniel 2013; Hudson, Sharma, and Vimalanathan 2009).

Although the water industry is the largest user of ozone gas, medical ozone, produced and applied under strict conditions, is an excellent therapy (Bocci 2002; Viebahn-Hänsler, León Fernández, and Fahmy 2016). The virucidal and bactericidal properties of ozone in health have been recognized since World War one, when ozone was applied to disinfect wounds and mustard gas burns (Viebahn-Hänsler 2007). If incorrectly applied, ozone can be toxic to humans, but when it is applied appropriately (Bocci 2002; Viebahn-Hänsler, León Fernández, and Fahmy 2012), ozone is effective to treat a multitude of diseases including wounds, ulcers, circulatory disorders, viral diseases (e.g., Ebola), and even cancers (Bocci 2002; Elvis and Ekta 2011; Rowen 2019). In fact, ozone is not a strange molecule to the human body since our immune system naturally produces it as part of its defense strategy against invading microbial pathogens (Loscalzo 2004; Wanjala et al. 2018; Wentworth et al. 2003). As part of its molecular defense network, salivary antibodies also catalyze ozone formation to kill microorganisms efficiently in the oral cavity (Fábián et al. 2012). The action mechanisms of ozone in the medical field include inactivation of microorganisms, stimulation of oxygen metabolism, and activation of the immune system (Elvis and Ekta 2011). As an oxidation therapy, medical ozone is administered through various routes including ozonized saline solution, autohemotherapy, extravascular blood oxygenation-ozonation, body exposure to ozone gas, rectal insufflation, etc. (Bocci, Zanardi, and Travagli 2011; Moreno-Fernandez et al. 2019; Schwartz 2016; Viebahn-Hänsler 2007). Methods that administer ozone directly to the infected part (e.g., by bagging or application of ozonated aqueous solutions on a wound) result in oxidative attack of the pathogens. However, when ozone is supplied to the blood, its direct virucidal activity is not guaranteed due to a protection provided by the antioxidant system in blood or the intracellular viruses become inaccessible to ozone. As ozone is transferred to blood, it instantly reacts with blood biomolecules such as unsaturated fatty acids to generate reactive oxygen species and lipid oxidation products such as hydrogen peroxide, ozonides, and lipid peroxides. These ozone reaction products will act as ozone messengers responsible for stimulating the biological and therapeutic effects of ozone instead of a direct attack of the viruses by ozone (Bocci 2006; Bocci et al. 1998; Zanardi et al. 2016). Ozone is toxic when breathed; thus it must not be inhaled.

Mode of action of ozone against viruses

Due to its high oxidizing property, ozone is particularly lethal against viruses, both enveloped and non-enveloped (Murray et al. 2008). Although the mode of action of ozone against viruses is yet to be fully clarified, ozone is likely to react with viruses through the direct molecular ozone reaction mechanism and/or indirectly through reactive oxygen species (ROS) such as $^{\cdot}OH$, $O_2^{\cdot-}$

and H_2O_2 produced as a result of ozone decomposition. In addition, the reactions between ozone and its ROS with the constituents of the virus structure including lipids, proteins, and amino acids could lead to the formation of other ROS including reactive radicals ($RCOO^{\cdot}$) which further propagate oxidation through a chain reaction. Ozone reacts readily with several biological compounds in the order of preference: lipids (particularly polyunsaturated fatty acids)>antioxidants>cysteine-rich proteins>carbohydrates (Bocci 2002). Murray et al. (2008) showed that ozone inactivates viruses through lipid and protein peroxidation followed by subsequent damage to the lipid viral envelope and protein capsid. Kim, Gentile, and Sproul (1980) showed that ozone (<1 mg/L for 5 seconds) inactivated bacteriophage f2 in water and have found that ozone attacked the phage coat, breaking the protein capsid into subunits while Roy et al. (1981) have concluded that damage to the viral nucleic acid was the major cause of poliovirus 1 inactivation by ozone. Genome attack by ozone has also been recently confirmed by Young et al. (2020). Thus, ozone and its ROS are capable to attack the virus at different sites of its structure destroying it and making it inactive to infect.

Potential inactivation of SARS-CoV-2 with ozone

SARS-CoV-2 is reported to belong to the β-B group of coronaviruses and has a diameter in the range 50 to 200 nm (Zhou et al. 2020) (approximately 500 times larger than the size of an ozone molecule (Table S1)). Similar to other coronaviruses, SARS-CoV-2 has a structure consisting of a core genetic material surrounded by an envelope of protein spikes resembling a crown (Xu et al. 2020a). Figure 1(a) shows a model representation of SARS-CoV-2 structure. The core genetic material is a single positive-sense RNA genome protected by a nucleocapsid (N), while the viral envelope is created by three structural proteins: spike (S), envelope (E), and membrane (M). The genome encodes these four major structural proteins (i.e., S, E, M, and N), which are all required to produce a structurally complete virus. The spike S-protein is made of two subunits S1 and S2 and is responsible for initiating the infection events through a strong binding of subunit S1 to the human angiotensin-converting enzyme 2 (ACE2) receptor (Xu et al. 2020a). The binding affinity of S1 to ACE2 was found to be 10 to 20 times higher than that of SARS-CoV (the virus that was responsible for the outbreak of SARS in 2003) (Wrapp

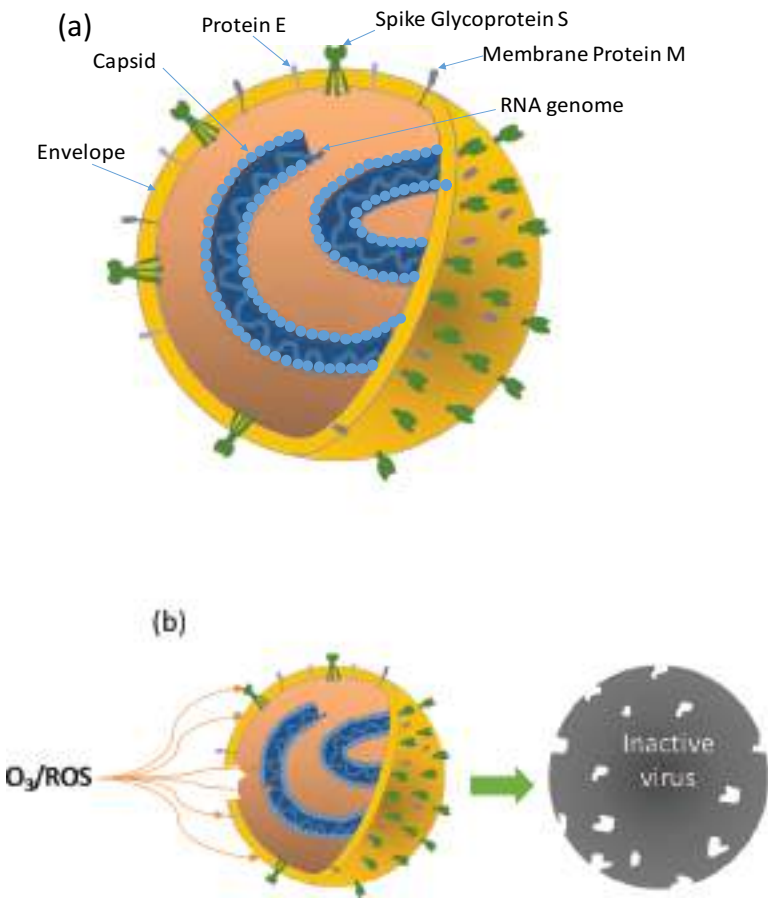

Figure 1. (a) Structure of SARS-CoV-2; (b) Potential sites for ozone attack leading to inactive virus.

et al. 2020), which could explain the high infectivity and transmissibility of SARS-COV-2 as compared to SARS-CoV. After binding of the subunit S1 to the receptor ACE2 on the host cell, the subunit S2 forms a six-helix bundle, serving to bring into close proximity the viral and cellular membranes for fusion and infection.

Ozone oxidation of the S-proteins could, therefore, inhibit the infection process. As shown in Figure 1(b), ozone and its ROS could also attack the envelope of the virus and if ozone and its ROS are able to penetrate the envelope, they could attack the genome capsid and its RNA making the virus unable to reproduce. Currently, there are no studies on ozone inactivation of SARS-CoV -2 but in a study conducted in China following the 2003 SARS outbreak, ozone was found effective to kill SARS-CoV virus in water within minutes (Jia-min et al. 2004). This suggests that ozone could be a potential lethal oxidant against SARS-CoV-2 since both viruses come from the same group and have similar structures; sharing 79.6% of the genome structure and having 89.8% identical sequence of the subunit S2 while the S1 subunit of both viruses bind to human ACE2 (Zhou et al. 2020).

The lipid structure of SARS-CoV-2 contains fatty acids, such as arachidonic acid (AA), linoleic acid (LA), palmitic (PA) and oleic acids (OA) (Yan et al. 2019) and is rich in glutamic acid (Cárdenas-Conejo et al. 2020). Particularly, AA and LA have been found to play an important role in the coronavirus infection mechanism (Yan et al. 2019). In addition, amino acids including cysteine and tryptophan are abundant molecules in the coronavirus proteins such as those in the spikes and the envelope and are crucial in membrane fusion (Broer et al. 2006). Besides, the amino acid methionine plays an important role in stabilizing protein structure and virus replication (Svancarova and Betakova 2018; Valley et al. 2012). The glycoproteins in the spikes, which promote entry into cells, are densely decorated by heterogeneous N-linked glycans and are the main target of antibodies (Walls et al. 2020). To evaluate the reactivity of ozone with molecules in the structure of SARS-CoV-2, these fatty acids and amino acids, in addition to representative N-glycopeptides (FSNVTWF (site N61) of spike protein subunit 1 (GS1) and EGVFVSNGTHWFVTQR (site N1098) of spike protein subunit 2 (GS2)) (Shajahan et al. 2020) have been modeled in Gaussian 19 using the semi-empirical PM6 method. Molecules of known reactivity with ozone in the order phenol > methyl benzene > benzene were also modeled for comparison purpose only using the same modeling method (i.e., PM6). The reactivity of ozone was evaluated by calculating the highest occupied molecular orbital (HOMO) energy values for each molecule; molecules with high HOMO react faster with ozone (Naumov and von Sonntag 2010).

Figures 2 and 3 show surface and bar chart plots of the HOMO energies of the studied molecules. Figure 3 shows that ozone has very high reactivity toward the amino acids tryptophan and methionine since they have the highest HOMO energies. According to Figure 3, the HOMO energies of tryptophan and methionine are comparable, indicating that they have similar reactivity with ozone, a result which is in agreement with Sharma and Graham (2010). The high reactivity of ozone toward tryptophan (even higher than phenol) is reasonable since the reaction takes place at the indole ring, which has high electronic density (Figure 2) while methionine's high reactivity is due to the sulfur-methyl thioether group. In their study of methionine oxidation, Choe et al. (2015) showed that methionine was preferentially targeted, with respect to other amino acids, forming predominantly methionine sulfoxide. Ozone reactivity is sensitive to the degree of ionization of a molecule, which is related to pH. In this study, the simulations were made considering neutral forms of the molecules but to illustrate the importance of pH, simulations were made allowing for the speciation of tryptophan, methionine, and cysteine at two pHs (7 and 8). At these pHs, the degree of ionization of tryptophan and methionine does not change significantly, with both molecules remain in their neutral forms by more than 95% (Figures S1, S2, and S3). However, the degree of ionization of cysteine changes significantly as pH changes from 7 to 8 as illustrated in Figure S4 and Table S2. When protonated at low pH, cysteine has three acidic hydrogens including carbonyl (-COOH), amino ($-NH_3^+$), and thiol (-SH). As the pH increases, cysteine looses hydrogen ions to solution according to the values of its dissociation constants (Eq. S3 and Figure S4). At the pHs 7 and 8, cysteine exists predominantly in its two forms AH_2 and AH^- at the percentages 94% (AH_2) and 6% (AH^-) at pH7 and 60% (AH_2) and 40% (AH^-) at pH8 (Table S2). The corresponding HOMO energies, calculated by Gaussian 16, of the species AH_2 and AH^- of cysteine, are −9.73 eV and −4.20 eV, respectively, indicating that the ionized form of cysteine (AH^-) is particularly very reactive with ozone since its HOMO energy is much higher than species AH_2. Considering weighted averages, cysteine HOMO energies are calculated and the values are −9.39 eV and −7.52 eV at pH 7 and 8, respectively (Table S2). Data from Naumov and von Sonntag show that for each unit increase of the HOMO energy, the rate constant of ozone reaction increases by approximately 4 folds (Naumov and von Sonntag 2010). Thus at pH8, cysteine is significantly more reactive than at neutral pH. Wolf, von Gunten,

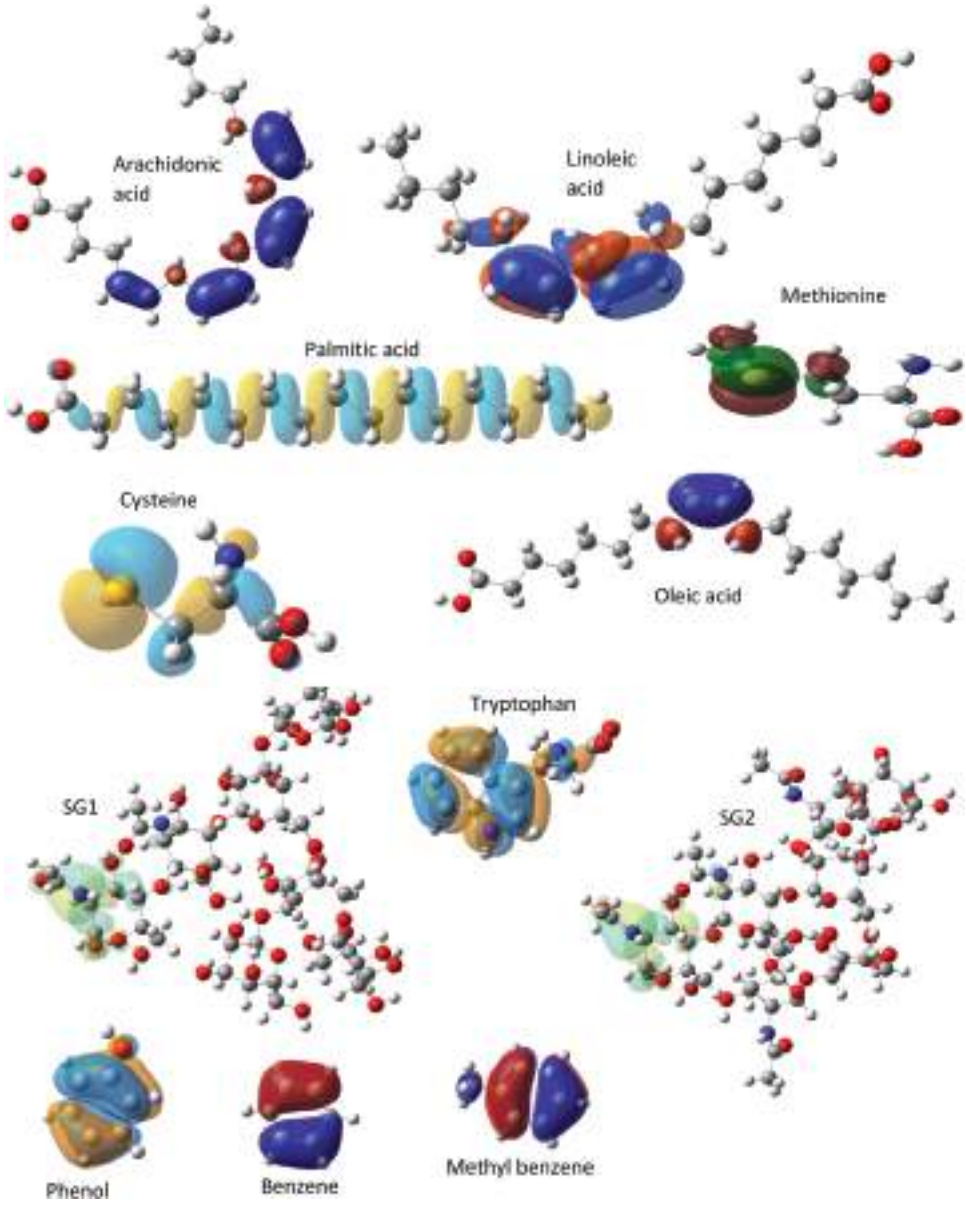

Figure 2. Computed surface plots of HOMO energy of molecules in SARS-CoV-2 structure. SG1: FSNVTWF (site N61) of spike protein subunit 1 and SG2: EGVFVSNGTHWFVTQR (site N1098) of spike protein subunit 2 according to Shajahan et al. (2020). Phenol, benzene, and methyl benzene are for comparison only.

and Kohn (2018) have also shown that ozone virus inactivation rate constants increase with increasing pH. On the other hand, both tryptophan and methionine have comparable HOMO energies of −8.81 eV and −8.83 eV, respectively, indicating that they have similar reactivity with ozone, which is in agreement with Sharma and Graham (2010). Comparing tryptophan and methionine to cysteine, both appear more reactive toward ozone at pH7 than cysteine since their HOMO energies are higher (Figure 3). However at pH8, due to ionization thus high HOMO energy, cysteine (−7.52 eV) becomes more reactive than tryptophan and methionine (Figure 3), which is in agreement with the results presented by Sharma and Graham (2010).

According to Figure 3 and among the fatty acids, arachidonic acid presents the highest reactivity toward ozone followed by linoleic acid, oleic acid, and palmitic acid. This is consistent with the number of unsaturated bonds (UB) in each fatty acid: AA (four UB), LA (two UB), OA (one UB), and PA (zero UB). The three fatty acids AA, LA, and OA have lower reactivity than phenol and methylbenzene but higher reactivity than benzene, while PA has the lowest reactivity due to the absence of unsaturated bonds in its molecular

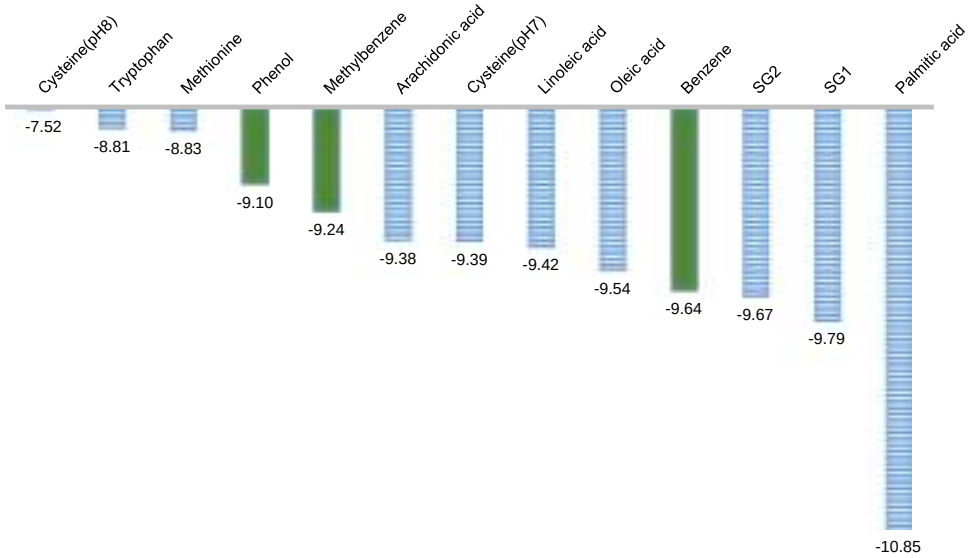

Figure 3. HOMO energy values (eV) for molecules in SARS-CoV-2 (blue) and comparative molecules (green).

structure. Cysteine is also prone to ozone attack at pH 7 with a reactivity higher than benzene but lower than phenol and methylbenzene. On the other hand, the spike protein subunit 2 (GS2) has similar reactivity to benzene while protein subunit 1 (GS1) has slightly lower reactivity, possibly due to the absence of double bonds in their molecular structures. Considering these results, ozone appears effective to attack the amino acids (particularly tryptophan, methionine, and cysteine (at pH8)) and fatty acids (particularly arachidonic acid), thus able to disrupt the protein and lipid structures of the virus, which could lead to its destruction. Since these molecules play key roles in the pathogenesis of coronaviruses (Broer et al. 2006; Yan et al. 2019), their disruption could also disrupt and inhibit key events in the viral infection mechanism such as virus binding and fusion with the host cell, thus ozone oxidation renders the virus not infective. Besides molecular ozone, radicals produced from its decomposition could cause further damage to the virus's structure, thereby increasing the likelihood of ozone efficacy against this deadly virus.

Conclusions

Through molecular modeling, this study showed that ozone could be an effective oxidant against SARS-CoV-2. Ozone could attack the proteins and lipids of the virus's spikes and envelope, thus destroying the integrity of the virus and inhibiting the mechanism by which it infects. Ozone could support the current effort in fighting COVID-19.

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