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Research Article

Inactivation of SARS-CoV-2 in the Liquid Phase: Are Aqueous Hydrogen Peroxide and Sodium Percarbonate Efficient Decontamination Agents?

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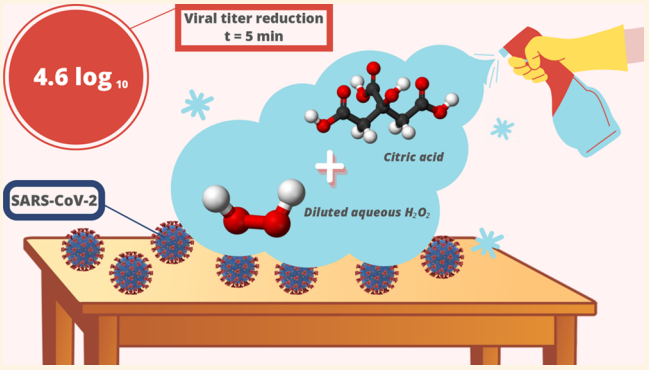
ABSTRACT: A diluted 3% w/w hydrogen peroxide solution acidified to pH 2.5 by adding citric acid inactivated SARS-CoV-2 virus by more than 4 orders of magnitude in 5 min. After a contact time of 15 min, no viral replication was detected. Aqueous solutions of sodium percarbonate inactivated coronavirus by >3 $\log_{10}$ diminution in 15 min. Conversely, H_2O_2 solutions with no additives displayed a scarce virucidal activity (1.1 $\log_{10}$ diminution in 5 min), confirming that a pH-modifying ingredient is necessary to have a H_2O_2-based disinfectant active against the novel coronavirus.



KEYWORDS: *COVID-19, SARS-CoV-2, hydrogen peroxide, sodium percarbonate, biocidal agents, chemical inactivation, disinfection, virucidal solutions*

INTRODUCTION

The coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) challenged the world for effective strategies for infection prevention and control, mitigation and containment, against virus dissemination.[1]

Preventive measures are currently the most effective strategy to limit pathogen diffusion, reduce the overload on the healthcare system, and isolate newly identified cases. Since person-to-person transmission mostly takes place through the emission of droplets from the upper respiratory tract during breathing, talking, sneezing, or coughing, it is essential to minimize the spread of such liquid particles, especially in confined environments, and to avoid the contamination of objects and surfaces likely to become a further source of infection.[2−4]

Social distancing and mandatory face coverings prevent and mitigate interhuman transmission, especially when coupled with lock-down actions, quarantine, contact tracing, and massive testing of suspects.[5,6] At the same time, special attention is paid to understanding coronavirus persistence on inanimate surfaces.[7] Several studies investigated the performance of simple chemical biocide agents for the safe inactivation and/or degradation of coronaviruses on surfaces and water.[8,9] Such indications were the basis for national and international health agencies to define adequate procedures for cleaning, sanitization, and disinfection of indoor spaces, objects, furniture, and tools in healthcare facilities as well as in daily household practice.[1,10] According to these guidelines, potentially contaminated surfaces are typically sprayed or wetted with large excess amounts of virucidal solution, and a few minutes of action at room temperature is necessary for a satisfactory viral inactivation (mainly, spanning from 1 to 15 min or until the sprayed solution evaporates).[10−12]

To date, the most widely adopted methods to inactivate coronaviruses, in particular SARS-CoV-2, on surfaces primarily rely on highly concentrated aqueous alcohol solutions (>70% w/w),[13,14] aqueous solutions of active chlorine-generating compounds,[15−17] and formulations containing quaternary ammonium cationic surfactants.[18]

WHO's guidelines suggest ethanol and 2-propanol for hand sanitization because of their rapid activity, broad-spectrum microbicidal effectiveness, cheapness, and reasonable safety.[19−21] Alcohols act on enveloped viruses denaturing the

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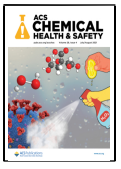

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ACS Chem. Health Saf. 2021, 28, 260−267

envelope viral proteins and disrupting its phospholipidic double layer.[22] However, during the first months of the COVID-19 emergency, some southern European countries suffered from a shortage in the availability of ethanol and 2-propanol, because of the sudden high demand on the local market, to be used in liquid and/or gel sanitizing formulations.[23] Also, the high percentage of highly flammable alcohols in disinfecting solutions (the flash point of a 70 vol % ethanol solution in water is 21 °C[24]) increased the risk of fires and explosions in facilities where large amounts of sanitizing solutions were stocked, and several accidents occurred in improvised warehouses within healthcare facilities.[25]

Sodium hypochlorite-based aqueous solutions at concentrations 0.1−0.5% inactivate coronaviruses with a >3.0 $\log_{10}$ reduction in viral titer within 1 min of contact time.[13,26] Undissociated hypochlorous acid, HOCl, as well as highly active oxidizing free radicals formed during the dissolution of these agents in water play indeed a key role in the antimicrobial activity. Hypochlorite-containing diluted aqueous solutions are nonflammable and safe, but they can generate chlorine-containing products, which pose a threat in terms of long-term toxicity to humans and the environment. Indeed, several concerns exist (in terms of negative environmental impact on biota in surface waters or disruption of wastewater treatment unit operations) on the extensive use of chlorine-based biocidal agents, especially for large-scale sanitizations.[18,27,28] Then, various biocides containing quaternary ammonium salts (also known as quats[29]) are included in the EPA's list N recommendation list: benzalkonium chloride, benzethonium chloride, methylbenzethonium chloride, or cetylpyridinium chloride.[30] However, research led to controversial results in coronavirus inactivation,[26] and their specificity on SARS-CoV-2 is still debatable.[31] Some authors, in fact, raised concerns on the environmental impact due to a massive use of quaternary ammonium agents, too.[32,33]

None of these virucidal formulations are free from drawbacks. Thus, a biocidal agent that is fast-acting, chlorine-free, intrinsically safe (in terms of flammability and toxicity to humans and environment), chemically stable, and cheap would attract major attention as a reliable countermeasure against the SARS-CoV-2 virus. Aqueous hydrogen peroxide is a suitable candidate for such formulations, as it is safe, especially at very low concentrations, does not give rise to hazardous or polluting byproducts (H_2O_2 breaks down over time into molecular oxygen and water), and is easily available as pharmaceutical grade solutions at 3% w/w concentration (also known as 10 volume hydrogen peroxide).[34]

In the present work, we evaluated the effectiveness of aqueous hydrogen peroxide solutions in the inactivation of SARS-CoV-2, controlling concentration, pH, and additives. A concentrated liquid suspension of the virus was treated with comparable volumes of diluted inactivating agent for a few minutes, in order to mimic the typical mode of action of a sanitizing solution on small amounts of infected body fluids. Two different aqueous hydrogen peroxide concentrations were tested, namely, ca. 3% w/w, as found in conventional commercial household disinfectants, and ca. 0.5% w/w, as described and studied in previous reports.[35,36] The commercially available adduct sodium carbonate−hydrogen peroxide, as the so-called sodium percarbonate ($Na_2CO_3{\cdot}1.5H_2O_2$), was also tested for its high stability and robustness over time, when in dry form, being suitable to efficiently replace unstable aqueous H_2O_2 solutions.

MATERIALS AND METHODS

A brief description of materials and methods is reported here. Further details can be found in the Supporting Information.

Two mother solutions of hydrogen peroxide at 6.64% ± 0.04 (w/w) and 1.02% ± 0.02 (w/w) were prepared diluting a 35.1% stabilized H_2O_2 solution with sterile ultrapure deionized water and stored at +4 °C until use. H_2O_2 content was determined by volumetric iodometric titration.

A sodium percarbonate (SP) aqueous solution was freshly prepared by dissolving $Na_2CO_3{\cdot}1.5H_2O_2$ in sterile ultrapure deionized water. The obtained mother solution at 1.00 ± 0.02% (w/w) was immediately used after measuring its H_2O_2 content.

The peroxide-containing aqueous solutions were mixed in a 1:1 ratio with the virus suspension, so that the final concentration of the biocidal agent was 50% of the pristine concentration. Thus, in the solutions named HP-3, HP-0.5, and SP, the peroxide contents were 3.32% w/w, 0.51% w/w, and 0.51−0.53% w/w, respectively. Uncertainties for these values were around ±0.02% w/w. A SARS-CoV-2 viral suspension was mixed with sterile distilled water (1:1 ratio) and used as the positive control of untreated virus.

Aqueous solutions of 0.187 M sodium carbonate decahydrate, 1.04 M citric acid, and 3.12 M acetic acid were prepared with sterile ultrapure deionized water and used to adjust the test media pH to the desired value.

HP-3 and HP-0.5 solutions at pH 7.3 and SP solution at pH 10.5 were used as such, without any addition of further pH-adjusting agents.

HP-3-C at pH 2.5 and HP-3-A at pH 3.6 were obtained by adding citric acid or acetic acid, respectively, to the HP-3 formulation. The potential presence of peroxoacids in HP-3-C and HP-3-A solutions was checked by differential ceriometric/iodometric titration, as described in ref 37 and by ^{13}C NMR analysis.

Contact times of 5, 10, and 15 min were chosen to simulate surface sanitizing conditions in common household practice. Each virucidal solution was tested in triplicate. At the end of contact time, a stoichiometric excess of catalase suspended in 50 mM potassium phosphate buffer at pH 7.0 was added to each test solution to decompose any residual H_2O_2 and stop the test. Either sodium carbonate or citric acid was added to adjust pH to a neutral level before the addition of catalase. The efficacy of the catalase action was confirmed by iodometric titration, and in all cases, no detectable amounts of residual H_2O_2 were found.

SARS-CoV-2 virus stock was prepared propagating the virus isolate (GenBank accession number: MW000351) in Vero E6 cells culture (see the Supporting Information).

The virus titer decay was evaluated using a plaque assay on Vero E6 cells for each experimental setting, and the results were compared with the positive control (see further details in the Supporting Information).[38] The same experimental protocol was used to investigate the possible cytopathic effect of catalase, citric acid, acetic acid, PC, and HP solutions, as single ingredients and in a mixture.

A quantitative real-time polymerase chain reaction, qRT-PCR, was performed to estimate viral RNA concentration of the untreated viral suspension and in samples with a significant after-treatment viral titer reduction. The final RNA concentration was expressed as copies per milliliter.

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ACS Chem. Health Saf. 2021, 28, 260−267

Table 1. Inactivation Performance against SARS-CoV-2 of Hydrogen Peroxide-Containing Aqueous Solutions

solution name	solution code	active ingredient	pH	H_2O_2 concentration (% w/w)	contact time (min)	virus titer[b] (pfu mL^{-1})
initial viral suspension[a]			7.3			1.4×10^6
hydrogen peroxide	HP-0.5	H_2O_2	7.3	0.51	5	1.2×10^6
	HP-3	H_2O_2	7.3	3.07	5	2.8×10^5
sodium percarbonate	SP	$Na_2CO_3 \cdot 1.5H_2O_2$	10.5	0.53	5	4.0×10^5

[a]Viral suspension used (SARS-CoV-2 dil. 1:1). [b]Plaque assay standard deviation ±0.2%.

Table 2. Inactivation Performance against SARS-CoV-2 of Hydrogen Peroxide-Containing Solutions under Various Conditions

solution name	solution code	active ingredient	pH	H_2O_2 concentration (% w/w)	contact time (min)	virus titer[b] (pfu mL^{-1})	qRT-PCR (copies mL^{-1})
initial viral suspension[a]			7.3			3.1×10^6	1.5×10^{11}
sodium percarbonate	SP	$Na_2CO_3 \cdot 1.5H_2O_2$	10.5	0.51	5	2.2×10^5	2.0×10^{11}
	SP	$Na_2CO_3 \cdot 1.5H_2O_2$	10.5	0.51	10	8.9×10^3	1.2×10^{11}
	SP	$Na_2CO_3 \cdot 1.5H_2O_2$	10.5	0.51	15	2.3×10^3	2.7×10^{10}
hydrogen peroxide	HP-3	H_2O_2	7.3	3.32	5	2.4×10^5	1.5×10^{11}
	HP-3	H_2O_2	7.3	3.32	10	2.1×10^4	1.4×10^{11}
	HP-3	H_2O_2	7.3	3.32	15	4.0×10^3	1.2×10^{11}
hydrogen peroxide + citric acid	HP-3-C	H_2O_2	2.5	3.31	5	6.8×10^1	8.0×10^{10}
	HP-3-C	H_2O_2	2.5	3.31	10	no viral replication	8.0×10^{10}
	HP-3-C	H_2O_2	2.5	3.31	15	no viral replication	5.8×10^{10}

[a]Viral suspension used (SARS-CoV-2 dil. 1:1). [b]Plaque assay standard deviation ±0.2%.

RESULTS AND DISCUSSION

The attention of this study was focused on the inactivation performance against SARS-CoV-2 shown by aqueous hydrogen peroxide solutions and the solid adduct hydrogen peroxide−sodium carbonate. The efficacy of the solutions in SARS-CoV-2 inactivation was evaluated by mixing each virucidal solution to a viral suspension and then testing the residual viral content by *in vitro* plaque assay on Vero E6 cells. Virus replication induces cell lysis, resulting in the formation of plaques on the cellular monolayer (Supporting Information, Figure S3). Such plaques can be then counted to quantify virus titer,[38] the difference between before and after treatment titers being proportional to the virucidal activity.

A virucidal solution is here considered effective against SARS-CoV-2 when at least a >3 $\log_{10}$ (>99.9%) diminution in the number of active viral units is recorded at the end of the treatment.[39,40]

Diluted aqueous solutions of hydrogen peroxide were first tested under conditions as close as possible to the ones reported in the previous literature,[26,35] namely, at concentrations of 0.5% and 3.0% as well as short treatment times of 5 min (Table 1). At the end of the testing time, the excess H_2O_2 was quenched by the addition of catalase (see the Supporting Information). A specific blank test was carried out to evaluate whether the presence of organic compounds in the culture media (DMEM medium with fetal bovine serum and penicillin−streptomycin additive) is able to lead to a diminution in the initial amount of H_2O_2 due to undesired redox reactions. Under the conditions tested, a maximum 2.5% decrease in hydrogen peroxide with respect to the expected initial content was observed, and the actual H_2O_2 concentrations reported in Tables 1 and 2 already take into account this intrinsic diminution.

Aqueous H_2O_2 failed to effectively inactivate the virus, the best result being a ca. 0.70 $\log_{10}$ reduction in 5 min, with a 3.07% w/w solution, which is comparable to common H_2O_2 household formulations found on the market. A 0.5% w/w hydrogen peroxide aqueous solution led to a negligible diminution of the virus titer in 5 min (1.2×10^6 vs 1.4×10^6). Such a result seems to be in contradiction with the efficacy data reported in some previous works, where a >4 $\log_{10}$ abatement was claimed after a contact time of 1 min.[26,35] In that case, an acidic formulation (pH 3.0) of hydrogen peroxide aqueous solution, specifically designed for the disinfection of hard surfaces,[41] showed an optimal virucidal activity against human coronavirus at concentrations as low as 0.5% w/w.[35] However, such a solution is a patented "accelerated" version of H_2O_2 in which proprietary additives (stabilizers, complexing agents, orthophosphoric acid derivatives, and pH buffers) are mixed to the active peroxide ingredient.[40,41] These additional ingredients play a relevant synergistic role in the inactivation of the coronavirus and positively affect the intrinsic virucidal capability of H_2O_2 itself.[40] Conversely, the results collected here are in line with previous observations, in which hydrogen peroxide solutions (pharmaceutical grade at 3.0% and 6.0% v/v concentrations) showed minimal virucidal activity for very short contact times (15 and 30 s), in particular when they are used as oral rinsing liquids. In that previous literature work, the authors observed a maximum 1.8 $\log_{10}$ virus titer reduction.[42] With regard to human coronaviruses, then, a very good virucidal activity (>4 $\log_{10}$ reduction) on surfaces was observed only for longer contact times (2−3 h) by application of H_2O_2 vapors, therefore, under far more drastic conditions[43] or in the presence of peroxyacetic acid as an additive.[44] The modest virucidal activity of diluted H_2O_2 was shown on other target viruses too, and very long contact times or higher concentrations are typically used to achieve satisfying virucidal activities.[45−48] Hydrogen peroxide displays in many cases a good biocidal activity, which is attributed to the *in situ* generation of hydroxyl radicals and other oxidizing oxygenated species reacting with lipids, proteins, and nucleic acids via several parallel mechanisms and hence disrupting the structure and the function of the biological pathogen.[49] Nevertheless,

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ACS Chem. Health Saf. 2021, 28, 260−267

Table 3. Inactivation Performance against SARS-CoV-2 in the Presence of Carboxylic Acids as Additives

solution name	solution code	active ingredient	pH	H_2O_2 concentration (% w/w)	contact time (min)	virus titer[b] (pfu mL^{-1})
initial viral suspension[a]			7.3			6.3×10^8
citric acid	C	no H_2O_2	2.5		5	1.0×10^8
acetic acid	A	no H_2O_2	3.6		5	1.5×10^8
hydrogen peroxide + acetic acid	HP-3-A	H_2O_2	3.6	3.07	5	1.6×10^5

[a]Viral suspension used (SARS-CoV-2 dil. 1:1). [b]Plaque assay standard deviation ±0.2%.

the use of high amounts of H_2O_2 (either in aqueous solutions, with high concentrations of it, or in vapor form, with very high local concentrations of active species) is often necessary to have a proper virucidal action, also in combination with high temperatures, additives, and coformulants.

The first set of results shown in Table 1, in which H_2O_2 showed a scarce effectiveness against SARS-CoV-2, prompted us to investigate if longer contact times and/or the presence of simple additives, in particular easily accessible pH modifiers, could have a positive effect on the solutions' performance.

Citric acid was chosen as an additive to lower the pH of the aqueous solution as it is a cheap, biologically compatible, and environmentally friendly acidifying agent. In addition, aqueous solutions of polycarboxylic acids, such as citric, malic, fumaric, malonic, and succinic acids, exhibited a high virucidal activity against enveloped viruses on their own, in the absence of other biocidal agents,[50] and have found practical applications as an additional ingredient in disinfectant solutions.[51,52]

On the other hand, in order to have a basic solution, the sodium carbonate−hydrogen peroxide adduct was chosen as a starting material, since it is a cheap and stable product that contains, at the same time, the active peroxide ingredient and the alkaline modifier. Its use as a biocidal or virucidal agent has been scarcely studied,[53−55] and only three products listed in US EPA's List N contain $Na_2CO_3 \cdot 1.5H_2O_2$ as an active ingredient.[30] Sodium percarbonate can be considered a more robust alternative to diluted hydrogen peroxide, as it can be stored for longer times and transported more easily without any significant loss in active ingredient. It finds applications in dry powder formulations for the oxidative decontamination and abatement of hazardous biological or toxic materials.[56−58]

In the second set of tests (Table 2), the presence of residual amounts of potentially virucidal agents (H_2O_2, citric acid, or sodium carbonate) was quenched by adding dosed amounts of catalase, sodium carbonate, or citric acid, respectively. Control tests were performed to confirm that *in situ* produced sodium citrate (pH 6.8 in the final mixture, after neutralization) did not show any virucidal activity on its own against SARS-CoV-2 or cytotoxic effects on Vero cells.

The initial 1:1 diluted untreated SARS-CoV-2 viral suspension showed a virus titer of 3×10^6 pfu mL^{-1}, with an RNA concentration of 1.5×10^{11} copies mL^{-1}; both virus and RNA values were high enough to appreciate a potential post-treatment decay (Table 2). The initial virus stock contains a virus titer far higher than the one found in biological fluid samples of an average infected patient.[59] The 1:1 mixture of the original virus suspension with the virucidal solution thus represents more drastic conditions than the ones found in real sanitization examples of surfaces contaminated by virus-containing droplets. In these cases, a large excess of disinfecting liquid is typically used over tiny amounts of deposited body fluids. Contact times in the range 5−15 min were then chosen, as they mimic the standard action time for most peroxide-containing virucidal formulations on objects.

A clear contact time-dependent viral titer reduction was observed for all aqueous solutions. With 3.32% aqueous H_2O_2, HP-3, at buffered 7.3 pH value, a gradual 1 log pfu mL^{-1} diminution was observed every 5 min of exposure to the disinfectant solution. This result is fully in line with the data reported in Table 1 for HP-3. A relatively long contact time (15 min) was thus necessary to obtain a ca. 2.9 $\log_{10}$ reduction of the viral titer in the absence of additives or accelerating coformulants.

On the contrary, the 3.32% H_2O_2 solution at pH 2.5, HP-3-C, showed the highest virucidal activity with more than 4.6 $\log_{10}$ pfu mL^{-1} reduction after only 5 min and a total virus elimination right after 10 min, so well beyond the threshold limit suggested by WHO for the disinfection of surfaces.[39] This is, to the best of our knowledge, the best virucidal performance recorded for diluted aqueous H_2O_2 solutions containing simple, nonpatented additives on viruses. The addition of citric acid to the disinfectant solution, not only modifies the pH of the final medium but also enhances the effect of hydrogen peroxide on the biological agent. Such a cooperative effect was previously observed in biocidal solutions, where short-chain carboxylic acids increase the biocidal action of the peroxide on various bacterial strains.[60] The *in situ* formation of monoperoxocitric acid could be hypothesized, under these conditions,[61] and such peroxoacid could play a key role in the inactivation of this coronavirus.[62]

It was thus necessary to ascertain the presence or absence of peroxocitric species, in the final liquid medium. By means of ^{13}C NMR spectroscopy, no differences were observed in the spectra of aqueous solutions of citric acid in the absence and in the presence of hydrogen peroxide, under conditions as close as possible to those used in virus inactivation tests. In particular, no changes were detected in the signals at 176.7 and 173.4 ppm, attributed to the two types of carboxyl groups of citric acid before and after the treatment with H_2O_2 (Supporting Information, Figures S1 and S2). Moreover, the absence of peroxocitric acid was confirmed by differential ceriometric/iodometric titration. Actually, the hydrogen peroxide content revealed by ceriometric method (that titrates H_2O_2, but not peroxoacids) was practically the same as the peroxide content measured by iodometric titration (method that is able to detect the presence of both hydrogen peroxide and peroxoacids).[37]

In order to establish whether carboxylic acids alone possess a virucidal activity against SARS-CoV-2, the viral suspension was mixed into pure solutions of citric acid (pH 2.5) and acetic acid (pH 3.6) (Table 3). Only a minimal diminution in viral titer was recorded (<0.8 $\log_{10}$) in the presence of the acids alone. On the contrary, a remarkable decrease of 3.6 $\log_{10}$ pfu mL^{-1} was observed in the presence of the solution HP-3-A, prepared by mixing hydrogen peroxide and acetic acid (in order to have pH 3.6). This virus inactivation value is comparable to the one observed with HP-3-C, with citric acid as an additive (Table 2).

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ACS Chem. Health Saf. 2021, 28, 260−267

Carboxylic acids are not able to satisfactorily inactivate the virus on their own. Rather, they are able to enhance the virucidal activity of H_2O_2 by lowering the pH value in the medium. In addition, the *in situ* formation of active peroxocarboxylic acids can be excluded, under the tested conditions.

In the presence of $Na_2CO_3{\cdot}1.5H_2O_2$, SP, a gradual diminution of ca. 1 $\log_{10}$ pfu mL^{-1} was observed every 5 min of contact time (Table 2). Nevertheless, the results obtained with SP are remarkable if one considers that the content of peroxide in SP solutions is only 1/6 of the amount present in HP-3-C solutions. In fact, the amount of $Na_2CO_3{\cdot}1.5H_2O_2$ was computed to have 0.51% w/w of H_2O_2 equivalents in the final liquid mixture vs ca. 3.0% w/w in the HP-3 and HP-3-C aqueous solutions prepared by diluting concentrated H_2O_2. This means that the actual inactivation capability of SP is very high, and the goal of >3 $\log_{10}$ of virus inactivation (in line with the WHO guidelines[39]) was successfully achieved after 15 min, also in the presence of a relatively less concentrated formulation. Actually, aqueous solutions of SP with ca. 3.0% of H_2O_2 equivalents cannot be prepared, as they are be beyond the solubility threshold of the solid in water. The good performance of aqueous SP solutions can be attributed not only to the oxidizing properties of H_2O_2 released by SP when dissolved in water but also to the highly alkaline environment (pH 10.5), caused by the presence of relatively high concentrations of Na_2CO_3 (0.683 M), which contribute in inactivating the virus by alkali-promoted degradation of its phospholipidic envelope.[63−65]

However, the excellent virus inactivation recorded for HP-3-C and SP was not observed in terms of residual RNA concentration: none of them showed a significant viral RNA diminution compared to the initial untreated viral suspension. Such an outcome was not unforeseen, since in several previous examples, H_2O_2 proved to be effective in virus inactivation, but with a modest or negligible degradation effect on viral RNA. The delicate interaction between the viral particle surface with its cellular receptor may easily be perturbed by a strong oxidant like H_2O_2. On the other hand, H_2O_2 itself has a less marked effect on "bare" nucleic acids, and typically the addition of catalytic amounts of metal centers (such as Fe(II) species, via Fenton-like reactions) is necessary to promote the extensive degradation of these substrates.[66−68] Moreover, the oxidative capability of the tested chemical compounds could have been partially depleted and weakened during the virus inactivation, making it less effective on nucleic acids. It is therefore likely that H_2O_2-containing solutions have a detrimental effect on the coronavirus envelope, thus inhibiting the virus' replication capability, without having a strong degradation effect on its genetic material. Therefore, high qRT-PCR values for RNA levels obtained from the liquid media after treatment with HP or SP do not necessarily reflect a scarce inactivation of SARS-CoV-2 virus and a reduction in its ability to infect target cells. Rather, they indicate that a large number of incompletely damaged short RNA sequences are still present in the culture medium after the inactivation process.

Finally, in order to avoid any misinterpretation of cell death due to undesired toxic effects by any of the ingredients of the disinfecting solutions, a control test was performed on a solution where H_2O_2 was completely disproportionated by catalase, and pH was brought back to a physiological value by the addition of a neutralizing agent. The absence of residual H_2O_2 and the final pH were confirmed by colorimetric cerium tests and pH spot measurements on the treated liquid solution. Interestingly, no cytotoxic effect was observed in any Vero cell culture treated with the peroxide-free and neutralized solutions. Therefore, in the present study, the cell monolayer disruption is solidly attributable to virus lytic ability rather than to a direct action of products or byproducts contained in the treated solutions.

CONCLUSIONS

Diluted aqueous solutions of hydrogen peroxide are often considered a poorly effective means of disinfection and biological decontamination. However, under acidic conditions and thanks to the addition of simple coformulants, H_2O_2 can be considered an active ingredient for the inactivation of SARS-CoV-2 virus. The copresence of citric acid to obtain an acid pH in the solution (ca. 2.5) and a minimum contact time of 5 min was a good combination to achieve a very good virus depletion with >4 $\log_{10}$ diminution. Such a result is the best performance recorded so far in the field of virus inactivation with diluted aqueous hydrogen peroxide liquid solutions.

Hydrogen peroxide with acetic acid, as an additive, proved to be an effective virucidal solution too, with a 3.6 $\log_{10}$ viral titer reduction in 5 min. In none of these cases, however, can the virus inactivation capability be attributed to the *in situ* formation of peroxocarboxylic acids.

The solid sodium carbonate−hydrogen peroxide adduct showed a less rapid inactivation capability, but in 15 min, a satisfactory reduction of more than 3 orders of magnitude in virus titer was attained.

H_2O_2-containing aqueous solutions displayed a limited direct degradation of the nucleic acid of the pathogen, since qRT-PCR target RNA sequences were found after the virucidal treatment. However, thanks to the addition of coformulants acting on the pH of the disinfecting solutions, liquid suspensions of SARS-CoV-2, with a virus concentration that is approximately 3−5 orders of magnitude higher than the one found in real biological fluid samples, were satisfactorily inactivated.

The promising results using acidified aqueous H_2O_2 diluted solution or with solid sodium percarbonate open the way to easily accessible, cheap, safe, robust, environmentally friendly, and efficient disinfecting agents to be applied in everyday-life sanitization practices and to be used by nonspecialized personnel too. Indeed, the use of diluted hydrogen peroxide modified, for instance, by the addition of common lemon juice, vinegar, or the use of sodium percarbonate for household cleaning purposes, may represent a viable and sustainable approach to obtain reliable virucidal solutions for a very broad range of the world population, also in countries with weaker economies. Further investigations are needed to show the effectiveness of these pH-modified aqueous H_2O_2 disinfectant formulations against other pathogens.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.chas.0c00095.

Expanded experimental details, ^{13}C NMR characterization, and *in vitro* plaque assay description (PDF)

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Notes

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