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3D REDUCED GRAPHENE OXIDE FILTERS DECORATED WITH SILVER NANOPARTICLES FOR RAPID REMOVAL OF SARS-COV-2 FROM WATER

FILTROS DE ÓXIDO DE GRAFENO REDUZIDO 3D DECORADOS COM NANOPARTÍCULAS DE PRATA PARA A REMOÇÃO RÁPIDA DE SARS-COV-2 DA ÁGUA

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ABSTRACT

Viral contamination in aquatic environments poses an ongoing challenge for water quality monitoring and environmental management, requiring efficient, scalable nanotechnological solutions for water treatment. In this study, we evaluate three-dimensional graphene-based nanofilters, with and without silver nanoparticle functionalization, for the removal of viral particles from water using SARS-CoV-2 as a model enveloped virus relevant to wastewater systems. The synthesized porous reduced graphene oxide (rGO) architectures all exhibited viral removal capacity. However, the silver and/or silver oxide-decorated material (10Ag-CTAB) showed the highest performance, achieving up to 99.1% efficiency in removing and retaining viral RNA signal from water at neutral pH and maintaining robust performance across acidic, neutral, and alkaline conditions, representative of natural waters and wastewater environments. The enhanced performance of 10Ag-CTAB can be attributed to the synergistic effects of the three-dimensional graphene structure and silver-based nanophases, which promote effective viral capture and retention. These findings demonstrate the potential of graphene-based nanofilters as a versatile and sustainable environmental nanotechnology for mitigating viral contamination in water treatment and management applications.

Keywords: Nanostructures; Silver nanoparticle; Nanofilters; Viral contaminants.

RESUMO

A contaminação viral em ambientes aquáticos representa um desafio constante para o monitoramento da qualidade da água e a gestão ambiental, exigindo soluções nanotecnológicas eficientes e escaláveis para

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o tratamento da água. Neste estudo, avaliamos nanofiltros tridimensionais à base de grafeno, com e sem funcionalização com nanopartículas de prata, para a remoção de partículas virais da água, utilizando o SARS-CoV-2 como um modelo de vírus envelopado relevante para sistemas de tratamento de águas residuais. As arquiteturas porosas de óxido de grafeno reduzido (rGO) sintetizadas apresentaram capacidade de remoção viral. Entretanto, o material decorado com prata e/ou óxido de prata (10Ag-CTAB) apresentou o melhor desempenho, atingindo até 99,1% de eficiência na remoção e diminuição do sinal de RNA viral da água em pH neutro e mantendo um desempenho robusto em condições ácidas, neutras e alcalinas, representativas de águas naturais e ambientes de águas residuais. O desempenho aprimorado do material 10Ag-CTAB pode ser atribuído aos efeitos sinérgicos da estrutura tridimensional do grafeno e das nanofases à base de prata, que promovem a captura e retenção viral eficazes. Esses resultados demonstram o potencial dos nanofiltros à base de grafeno como uma nanotecnologia ambiental versátil e sustentável para mitigar a contaminação viral em aplicações de tratamento e gestão de água.

Palavras-chave: *Nanoestruturas; Nanopartículas de prata; Nanofiltros; Contaminantes virais.*

1 INTRODUCTION

Viruses and other biological pathogens are frequently detected in surface waters, drinking-water sources, and wastewater, posing risks to human health and agricultural sustainability (Bleotu *et al.*, 2024; Carmona-Vicente *et al.*, 2024; Toribio-Avedillo *et al.*, 2023). Viruses transported by water, including enteric viruses, noroviruses, SARS-CoV-2, influenza, and related agents, are associated with several outbreaks ranging from gastrointestinal illness to respiratory infections, and their recurring detection in wastewater highlights the need for improved control strategies in water systems (Adriaenssens *et al.*, 2018; Ahmed *et al.*, 2023; Dlamini *et al.*, 2024). In parallel, phytopathogenic microorganisms in irrigation water can damage crops and reduce yields, underscoring the relevance of pathogen mitigation for food security and economic stability (Bačnik *et al.*, 2020; Lopez-Roblero *et al.*, 2024). In this sense, the development of effective approaches to reduce pathogen levels and molecular markers in water, including those associated with emerging threats such as SARS-CoV-2, remains a priority for public and environmental health protection (Alahdal *et al.*, 2023).

Nanomaterials have been explored as tools for water treatment due to their high surface area, mechanical properties, and modulable surface chemistry, which can be leveraged to enhance contaminant removal from water (Al-Hazmi *et al.*, 2023; Ebrahimi *et al.*, 2024; Ma *et al.*, 2024; Samriti *et al.*, 2023; Silvanir *et al.*, 2024; Yu *et al.*, 2021). Nanocarbon-based materials have attracted attention because their functional groups and porous architectures can facilitate interactions with biological particles in aqueous matrices (Ebrahimi *et al.*, 2022; Lavorato and Fontanova, 2023). The COVID-19 pandemic further intensified interest in technologies capable of mitigating viral contamination in water: SARS-CoV-2 has been repeatedly detected in wastewater and can persist for days, supporting the use of wastewater-based surveillance while motivating strategies to reduce viral charge in affected waters (Ansari *et al.*, 2024; Dargahi *et al.*, 2022; Graham *et al.*, 2021; Koza *et al.*, 2024; Sherchan *et al.*, 2023; Silva *et al*., 2022; Yuan *et al*., 2021). Potential downstream effects also warrant consideration, as viral components, such as spike protein fragments, have been linked to biological effects in aquatic biota (Charlie-Silva *et al*., 2021).

Graphene-based nanocomposites have shown promising antiviral properties, including graphene oxide (GO), which has been reported to capture SARS-CoV-2 in liquid media and inhibit infection under specific conditions, while also exhibiting bactericidal and biocompatible properties (de Maio *et al*., 2021). Fullerenes, carbon dots, graphene, and their derivatives have also been investigated for antiviral activity and comparatively low toxicity, with three-dimensional graphene structures standing out as attractive candidates for scalable filtration media (Chowdhury *et al*., 2025; Guha and Chakrabarty, 2025; Serrano-Aroca *et al*., 2021, Zhang *et al*., 2024). Incorporating silver species can further enhance performance by combining the high and versatile graphene surface versatility with silver established antimicrobial activity, motivating the development of silver-doped graphene platforms for water treatment applications (Anwesha *et al*., 2024; Dixit *et al*., 2024; Kim *et al*., 2012). In vitro studies have also shown that GO-based nanocomposites decorated with silver nanoparticles can inhibit viral replication and reduce extracellular viral release in coronavirus models (Cymerys *et al*., 2024).

Despite these advances, most studies involve dispersions, coatings, or biological endpoints under narrowly defined conditions, and few address real stand-alone, scalable filtration media designed to operate as engineered three-dimensional architectures in aqueous environments. In this work, we presented some porous, monolithic, three-dimensional reduced graphene oxide nanofilters and systematically compared rGO structures with and without Ag/Ag_2O decoration to clarify how the 3D architecture and silver-based nanophases influence the capture and retention of the SARS-CoV-2 marker in water. The term nanofilter refers to a porous nanostructured filtration medium composed of 3D-rGO-based materials, in which viral removal is primarily governed by adsorption and retention processes rather than by classical membrane nanofiltration mechanisms. By using RT-qPCR as the quantitative readout, we evaluated the performance of our materials across different pH values simulating those encountered in natural waters and wastewater environments. We identified a composition that rapidly and efficiently reduces the SARS-CoV-2 signal, supporting scalable graphene-based filtration platforms for water treatment and environmental monitoring.

2 EXPERIMENTAL

2.1 MATERIALS

Graphite (Sigma-Aldrich, < 20 μm), H_2SO_4, $KMnO_4$, H_2O_2 (30%), HCl 10%, ethanol, acetone, cetyltrimethylammonium bromide - CTAB (ACROS, 99% purity), ascorbic acid (Synth, 99% purity), silver nitrate (Neon PA/ACS), and deionized water.

2.2 PREPARATION OF GRAPHENE OXIDE

The first part of the synthesis was preparing graphite oxide (Gr-O) through a modified graphite oxidation method (Mehl *et al*., 2014). Initially, graphite was oxidized with H_2SO_4 and $KMnO_4$, followed by stopping the reaction with H_2O_2 and successive washes to obtain graphite oxide (Gr-O). Then, the Gr-O was exfoliated using ultrasound bath (Cristofoli, frequency 50/60 Hz, power 170 W), producing GO.

2.3 SYNTHESIS OF NANOFILTERS BASED ON THREE-DIMENSIONAL GRAPHENE AND SILVER PARTICLES

Different synthesis approaches were used to produce nanofilters with varying reagent concentrations, yielding nanomaterials with distinct characteristics (da Rosa *et al*., 2025; Leão *et al*., 2022; Matos *et al*., 2022). A total volume of 50 mL was used to prepare the nanocomposites. In 100 mL breakers, 50 mL of the GO dispersion (1.1 mg mL^{-1}) was added, followed by the addition of ascorbic acid as a reducing agent (10 mmol L^{-1}). To decorate 3D graphene with silver nanoparticles, silver nitrate was used as a precursor at concentrations of 10 or 30 mmol L^{-1}. In some variations, the cationic surfactant CTAB (1 mg mL^{-1}) was added as a passivant. A variation without the addition of $AgNO_3$ and CTAB was also obtained for control purposes. The beakers containing all systems were autoclaved for 90 min at 120 °C and 3.5 bar. The nanomaterials obtained were removed, washed, filtered with distilled water at approximately 80 °C, and placed in an oven at 60 °C until dry.

The different materials were named as follows: 3D-rGO for the silver-free nanofilter, composed solely of a three-dimensional reduced graphene oxide network. The nanofilters that were added only GO, ascorbic acid, and $AgNO_3$ were named 10Ag and 30Ag, indicating $AgNO_3$ concentrations of 10 and 30 mmol L^{-1}, respectively. The nanofilters with CTAB were named 10Ag-CTAB and 30Ag-CTAB, indicating the presence of 10 and 30 mmol L^{-1} of $AgNO_3$, respectively, plus 1 mg mL^{-1} of CTAB. The summary of the reagents used for each material preparation is shown in Table 1. All materials containing the abbreviation Ag correspond to 3D-rGO nanofilters decorated with silver particles, prepared under different synthesis conditions.

Table 1 - Quantity of reagents used for the synthesis of each material.

	$AgNO_3$ (mmol L^{-1})	**CTAB (mg mL^{-1})**
3D-rGO	-	-
10Ag	10	-
30Ag	30	-
10Ag-CTAB	10	1
30Ag-CTAB	30	1

2.4 CHARACTERIZATION OF NANOFILTERS

Scanning Electron Microscopy (SEM) images of the surfaces of the three-dimensional graphene samples were obtained using a Tescan MIRA3 FEG-SEM microscope with an In-beam detector. The source voltage was 10 kV.

2.5 STUDY OF THE ADSORPTION AND REMOVAL OF SARS-COV-2

Batch adsorption studies to evaluate the efficiency of adsorbent materials in removing SARS-CoV-2 were carried out according to the method of Yang and Tang (2016), with approximately 20 mg of adsorbent placed in 15 mL test tubes containing 5 mL of the aqueous solution containing the attenuated virus. The procedure was repeated for the five different nanofilters (3D-rGO, 10Ag, 30Ag, 10Ag-CTAB, and 30Ag-CTAB). The pH values of the solutions were 5, 7, and 9, representative of sewage, potable water, and wastewater, respectively. The flasks were shaken on a shaker table at 200 rpm and 25 °C for 24 h.

2.6 VIRUS REMOVAL EXPERIMENT

An inactivated SARS-CoV-2 virus is used as a positive control and is derived from a clinically isolated Vero-E6 cell culture (SARS-CoV-2/SP02/human2020/Br, GenBank accession number MT126808.1). This virus was kindly provided by Prof. Dr. Edison Luiz Durigon from the Department of Microbiology, Institute of Biomedical Sciences, University of São Paulo (USP), Brazil (Dorlass *et al.*, 2020).

2.7 REMOVAL OF SARS-COV-2 FROM THE WATER

10 mg of each adsorbent were properly dried at 37 °C for 2 h. Afterward, the adsorbent was transferred to a microtube containing 1.5 mL of ultrapure water (free of all RNAse enzymes), and 150 µL of the inactivated SARS-CoV-2 viral suspension (2.5 × 106 copies/mL) was then added, which was followed by incubation with shaking at 200 rpm and 28 °C for 24 h. Subsequently, the supernatant and adsorbent were removed and transferred to another microtube, and viral RNA was extracted. The virus adsorption was calculated and presented as described by Demarco *et al.* (2022).

CT values are inversely related to viral load and are an indirect measure of viral RNA copy number (Rao *et al.*, 2020).

2.8 RNA EXTRACTION

The RNA was extracted from both supernatant and the studied adsorbents using a MagMax™ Core Nucleic Acid Purification kit (Thermo Fisher Scientific, Waltham, MA, USA). The extracted RNA was quantified by Nano Drop® (Thermo Scientific, Waltham, MA, USA). Approximately 10 ng of RNA was used for RT-qPCR.

2.9 QRT-PCR

The primer and probe used in PCR reactions were designed based on sequences published by the Centers for Disease Control and Prevention (2020). Briefly, a reaction of 25 μL of final volume was used, with the following volumes added to the 1 × concentrated master mix: 5 μL of sample RNA, 12.5 μL of 2 × reaction buffer, 1 μL of Superscript™ III One-Step with Platinum™ Taq DNA Polymerase (Invitrogen, Darmstadt, Germany), 0.4 mM of each dNTP, 0.4 μL of a 50 mM MgSO4 solution (Invitrogen), 1 μg of non-acetylated bovine albumin (Roche), 10 μM of each primer 2019-nCoVN1-F2019-nCoV N1 (5′GACCCCAAAATCAGCGAAAT3 ′), 2019-nCoVN1-R2019-nCoV N1 (5′TCTGGTTACTGCCAGTTGAATCTG3′), 2019-nCoVN1-P2019-nCoV N1 probe (5′-FAM - ACCCCGCATTACGTTTGGTGGACC- BBQ 3′), and DEPC water. The reaction occurred in StepOne™ Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA, USA) in the following cycling: 55 °C for 10 min for reverse transcription, followed by 95 °C for 3 min and 40 cycles of 95 °C for 15 s, 58 °C for 30 s.

2.10 STATISTICAL ANALYSIS

Data were expressed as mean ± standard deviation for duplicates for each experimental point. Data were analyzed by using one-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison tests adjusted for a significance level of 5%.

3 RESULTS AND DISCUSSION

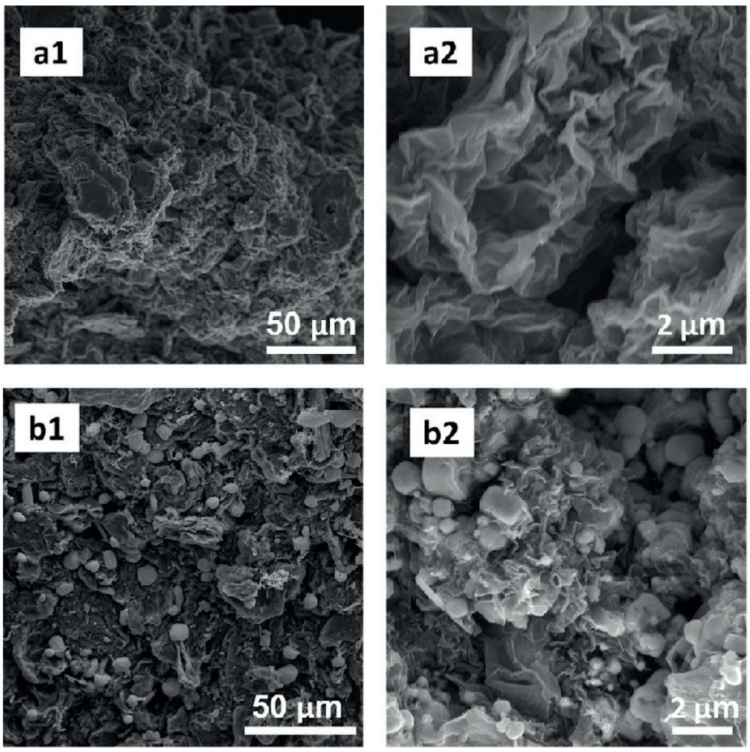
3.1 NANOFILTERS MORPHOLOGY

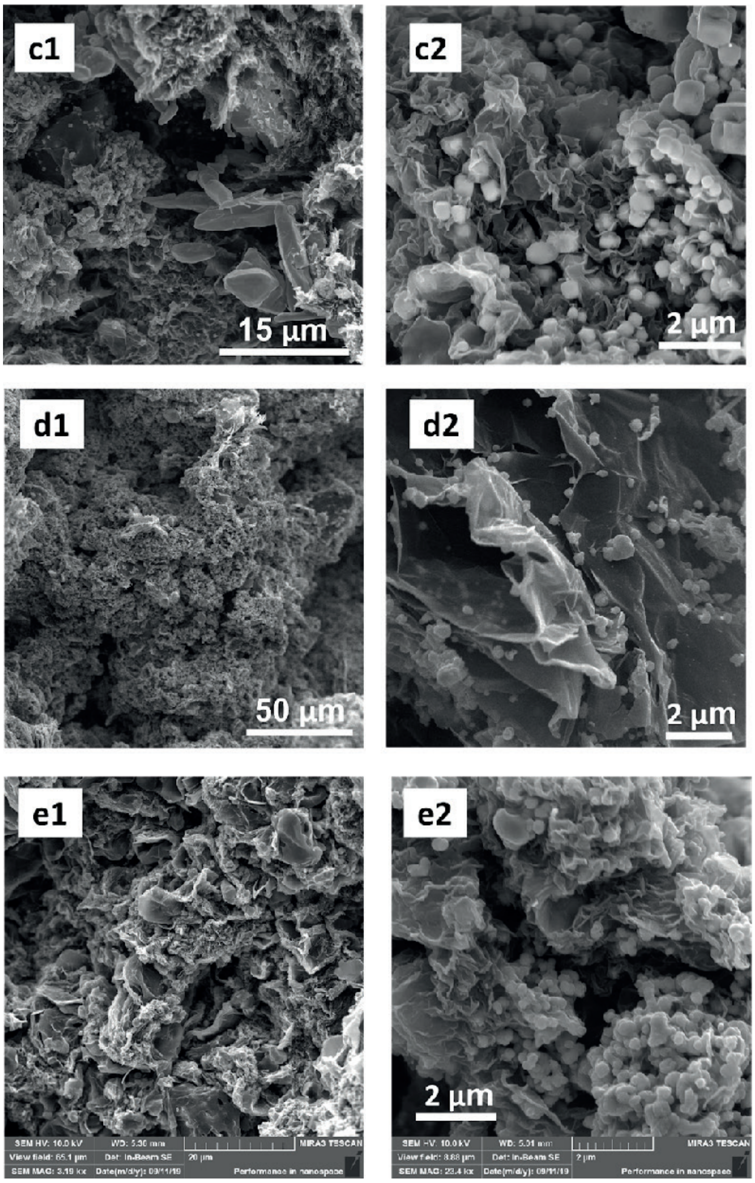
Figure 1 shows the SEM images of the samples: (a1-a2) 3D-rGO, (b1-b2) 10Ag, (c1-c2) 30Ag, (d1-d2) 10Ag-CTAB and (e1-e2) 30Ag-CTAB. In the 3D-rGO material (Figure 1a), without silver particles, the formation of the three-dimensional structure of reduced graphene oxide (rGO) sheets gives rise to the porous macrostructure based on rGO, which has pores of different sizes. It is observed that the sample without nanoparticles (Figure a1-a2) presents micro- and nanometric pores. In the samples containing silver, the main morphological difference lies in the absence or presence of CTAB. In the absence of CTAB, particle size heterogeneity is greater, with some particles reaching micrometric dimensions (Figure b1-c2). The presence of CTAB reduces nanoparticle size and improves their homogeneous distribution throughout the material. (Figure d1-e2).

The synthesis, characteristics, and morphology of these materials are described in detail by Leão *et al.* (2022). The materials containing silver particles are described in detail by da Rosa *et al.* (2025). In the same previous work, we reported that regarding the chemical nature of the particles, in samples containing only metal, the particles are composed solely of metallic silver. However, in the presence of CTAB, silver oxide is observed in addition to metallic silver. The Ag_2O: Ag ratio is higher in samples prepared with a lower silver precursor content.

In Rosa *et al.* (2025), SEM analyses demonstrated the successful formation of silver-containing nanoparticles anchored to the 3D-rGO framework. Furthermore, the nanoparticle size and distribution were strongly influenced by the synthesis conditions. The addition of CTAB after the silver precursor promoted the formation of smaller, more uniformly distributed nanoparticles, whereas increasing the AgNO3 concentration from 10 to 30 mmol L^{-1} led to particle growth and agglomeration. XRD analyses further confirmed the presence of Ag and Ag_2O phases in the decorated materials. Among the synthesized nanofilters, the 10Ag-CTAB sample exhibited the smallest nanoparticle size and the most homogeneous distribution, characteristics that may contribute to its superior viral removal performance observed in the present study.

Figure 1 - SEM images of 3D-graphene based materials samples
(a1-a2) 3D-rGO, (b1-b2) 10Ag, (c1-c2) 30Ag, (d1-d2) 10Ag-CTAB and (e1-e2) 30Ag-CTAB.

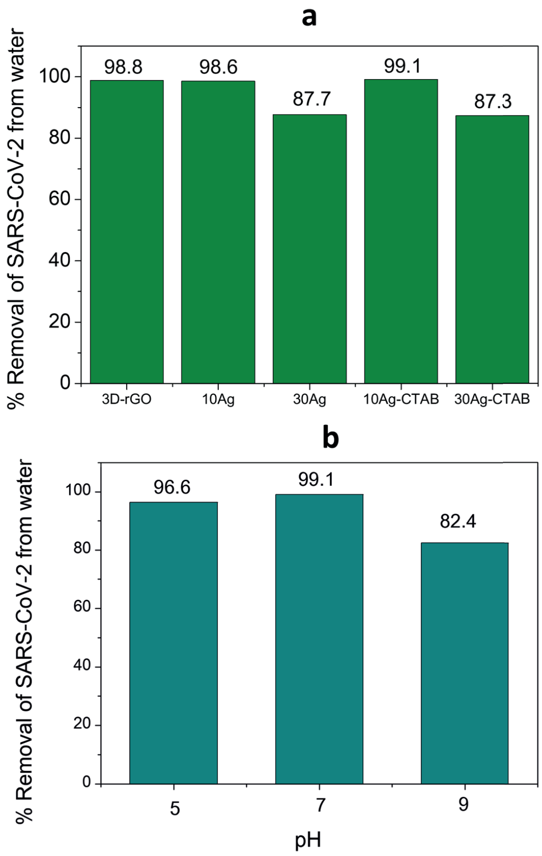




3.2 REMOVAL OF SARS-COV-2 FROM THE AQUEOUS SOLUTION

The viral load removal percentage of SARS-CoV-2 from the aqueous solution was determined by RT-PCR and is shown in Figure 2. Firstly, removal results were obtained for all materials (3D-rGO, 10Ag, 30Ag, 10Ag-CTAB and 30Ag-CTAB) at pH 7, as shown in Figure 2a. Furthermore, it was also possible to obtain the removal percentage for the 10Ag-CTAB material at three different pHs (5, 7 and 9), shown in Figure 2b. Recently, it has been shown that graphene-based nanoplatforms exhibit strong antiviral activity against SARS-CoV-2 (Donskyi *et al.*, 2021); however, studies demonstrating the efficiency of SARS-CoV-2 removal from water by graphene-based nanomaterials have not yet been reported in the literature.

Figure 2 - Percentage of viral load removal of SARS-CoV-2 from aqueous solution (a) for all materials (3D-rGO, 10Ag, 30Ag, 10Ag-CTAB, and 30Ag-CTAB) at pH 7 and (b) for the 10Ag-CTAB material at three different pHs (5, 7 and 9).



The 3D-rGO without nanoparticles material showed the second-best efficiency for removing viral load at pH 7 (Figure 2a), indicating that neat rGO sheets are excellent for anchoring and adsorbing SARS-CoV-2. Add to the porosity and possible high surface area of these materials, which allows for excellent filtration of water contaminated with this virus. Graphene can act as a nanoplatform, enabling surface functionalization of viruses or bacteria and promoting their adsorption, as it has a high binding Affinity (Anand *et al.*, 2019; Donskyi *et al.* 2019; Donskyi *et al.*, 2021; Nie *et al.*, 2019).

The mechanisms of interaction between graphene species and pathogens remain poorly understood (Shi *et al.*, 2016; Zou *et al.*, 2016). However, some suggested mechanisms include trapping pathogens through interactions with antibodies or specific ligands (Thomas *et al.*, 2020; Losada-Garcia *et al.*, 2020), electrostatic interactions (Gholamo *et al.*, 2017), trapping (Merino *et al.*, 2020), wrapping (Qi *et al.*, 2015) destruction of pathogens by hydrophobic (Donskyi *et al.*, 2019) or mechanical interactions (Ye *et al.*, 2015), disruption of the peptidoglycan membrane structure, entrapment of microorganisms, electron transfer and induction of oxidative stress by reactive oxygen species (ROS), as well as the combination of these mechanisms (Serrano-Aroca *et al.*, 2021; Tegou *et al.*, 2016; Zou *et al.*, 2016).

Graphene and GO can destroy virus surface proteins and extract their RNA by bioreduction (Guha *et al.*, 2025; Song *et al.*, 2015). Thus, a porous macrostructure consisting of a three-dimensional graphene network can interact with the SARS-CoV-2 structure and with rGO sheets for virus removal and inactivation, especially when decorated with silver particles and surfactant molecules (Carvalo and Conte-Junior, 2021; Jahromi *et al.*, 2020; Jeremiah *et al.*, 2020; Mosidze *et al.*, 2025).

When decorating the macrostructures with silver particles, in the case of 10-Ag and 30-Ag materials (Figure 2a), a slight decrease in removal efficiency is observed for the 10-Ag material and a more significant decrease for the 30-Ag material. Such a reduction may be related to particle size, as some particles are micrometer-sized, decreasing surface area, especially in the 30-Ag material, where most particles are micrometer-sized (Figure d1-d2). This reduction in surface area may explain the lower SARS-CoV-2 removal efficiency observed for these materials. However, the presence of silver particles increases the likelihood of virus inactivation (Carvalo and Conte-Junior, 2021; Jahromi *et al.*, 2020; Mosidze *et al.*, 2025). Therefore, future studies will examine the ability of these materials to inactivate the virus, particularly those decorated with silver particles.

The best SARS-CoV-2 removal efficiency from the aqueous solution at pH 7 was for the 10Ag-CTAB material (Figure 1 d1-d2). This result is probably associated with its smallest silver particle size distribution, preferably nanometer-sized, which maintains the high surface area of the macrostructure and allows for more significant interaction with the virus molecules. This particle size was obtained using the CTAB surfactant and the synthesis method (da Rosa *et al.*, 2025). As for the 30Ag-CTAB material (Figure 2a) there is a decrease in removal efficiency, but also an increase in particle size (Figure 1 e1-e2). Even so, the presence of silver and CTAB can increase the chances of inactivation of the vírus (Jeremiah *et al.*, 2020; Thomas *et al.*, 2020).

The 10Ag-CTAB material obtained better viral load removal at pH 7 and showed promising results at different pH values, as shown in Figure 2b. However, for the 10Ag-CTAB material, the optimal pH for viral load removal is neutral, followed by acidic pH, and the least efficient is basic pH. Even so, the average removal for 10Ag-CTAB across different pH values remains promising.

Ag_2O may be more effective than metallic silver in inactivating SARS-CoV-2 due to its higher release of Ag^+ ions, which are primarily responsible for antiviral activity. In addition to being more soluble, Ag_2O exhibits greater chemical reactivity, facilitating interactions with viral proteins and the virus's genetic material. Studies indicate that nanomaterials containing Ag_2O exhibit higher toxicity against viruses and bacteria, making them a promising option for antimicrobial coatings and filters, especially because they enable controlled, prolonged release of silver ions (Gudkov *et al.*, 2022; Rodrigues *et al.*, 2024).

4 CONCLUSIONS

In this work, porous three-dimensional reduced graphene oxide (3D-rGO) nanofilters, decorated with or without silver-based particles, demonstrated a high capacity for removing SARS-CoV-2 viral RNA signal from aqueous solutions. Among the evaluated materials, the 10Ag-CTAB nanofilter exhibited the best performance, achieving up to 99.13% removal efficiency at neutral pH. The superior performance of this material is attributed to the synergistic interaction between the three-dimensional graphene framework and silver-based nanophases, which enhance viral capture and retention. Furthermore, 10Ag-CTAB maintained high removal efficiency across a broad pH range representative of natural waters and wastewater, highlighting its potential for practical environmental applications. These findings demonstrate that silver-decorated 3D-rGO nanofilters are promising materials for mitigating viral contamination in water treatment systems. Future studies should investigate the mechanisms underlying viral retention and the potential of these nanofilters to inactivate viruses.

CONSENT FOR PUBLICATION

All authors have read and agreed to the published version of the manuscript.

AVAILABILITY OF DATA AND MATERIAL

Data will be available on request.

COMPETING INTERESTS

The authors have no relevant financial or non-financial interests to disclose.

AUTHOR CONTRIBUTIONS

PCCR: Methodology, Formal Analysis, Investigation, Writing. MBL: Formal Analysis and Article review, VSB Methodology, Formal Analysis, Investigation, Writing NLB Visualization, Writing, JGL: Formal Analysis and Article review JRB, Supervision and Article review; RAV: Conceptualization, Supervision, Investigation, Article review, CFMJ: Conceptualization, Supervision, Investigation, Article review.

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ETHICS APPROVAL

Not applicable.

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