


ARTICLE

Prevention of pathogen microorganisms at indoor air ventilation system using synthesized copper nanoparticles

Karine Machry¹ | Clóvis W. O. de Souza² | Mônica L. Aguiar¹ | André Bernardo¹ 

¹Chemical Engineering Department,
Federal University of São Carlos, São
Carlos, Brazil

²Microbiology and Parasitology
Department, Federal University of São
Carlos, São Carlos, Brazil

Correspondence

André Bernardo, Chemical Engineering
Department, Federal University of São
Carlos, São Carlos, São Paulo, Brazil.
Email: abernardo@ufscar.br

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Abstract

This article describes the impregnation of copper nanoparticles (CuNP) in a polyester fibre filter that can be used in solid–gas filtration to retain the spread of pathogen microorganisms in indoor environments. The impregnation of the CuNP was achieved by spraying the suspension on the surface of filter media. An acid pretreatment was also evaluated to increase the adhesion between fibre and nanoparticle. The synthesis of the CuNP was done by chemical reduction. The bacterial effect was measured through the contact method for *Escherichia coli* and *Staphylococcus aureus*, and we demonstrate that the presence of CuNP to filter media reduced up to 99.99% of gram-negative and 99.98% of gram-positive bacteria. The pretreatment with HCl was a good alternative to filter modification due to the higher adhesion between CuNP and the fibre while the high efficiency against pathogen microorganisms was kept. The modification of filters with CuNP can improve the air quality of indoor environments, vanishing the pathogen microorganisms circulating in the air.

KEYWORDS

adhesion of nanoparticles, air filtration, bactericidal effect, CuNP

1 | INTRODUCTION

The current pandemic caused by the new coronavirus (SARS-CoV-2) has increased the demand for new products with antibacterial and antiviral effects, and has become an important research topic.^[1–5] Nanoparticles are widely used against pathogens, especially silver nanoparticles as an agent against microorganisms.^[5,6] However, the cost of this precious metal can increase the price of the final product, such as masks or clothes, tailoring access to wealthier people. The global market of precious metals shows that the cost of copper is considerably lower than silver, gold, platinum, and even palladium.^[7] Copper is also a material widely known due to its electronic conductivity and also due to its fungicidal effect, used in agriculture, and has been widely explored as an agent against bacteria and viruses.^[8,9]

Filter media are the first physical barrier between a contaminated environment, such as an outdoor environment, and other clean environments, such as an indoor environment. In indoor environments, filters from ventilation systems, such as air conditioning, protect the indoor room from external agents, including dust, smoke, and pathogens. The maintenance of those filters is essential to enhance the air quality of indoor environments. This first barrier can also avoid the transmission of airborne pathogen microorganisms, such as influenza virus, Ebola, chickenpox, rubella, severe acute respiratory syndrome (SARS), Middle East respiratory syndrome (MERS), or even tuberculosis. The periodical study of the World Health Organization that reports the principal causes of death around the world showed (in 2017) that lower respiratory infections occupied

the fourth position for most frequent causes of deaths.^[10] These diseases include tuberculosis, pneumonia, and others, which can be aggravated due to bad air quality. The coronavirus pandemic (SARS-CoV-2) also reinforced the attention to respiratory infections and the air quality of indoor rooms, acting as a reminder of the importance of maintenance of these filters.

Recent studies proposed a route with UV-C light as a way to contain the spread of viruses in indoor spaces.^[2] As in masks, filter media corresponds to the first barrier between a contaminated environment, such as a room with a contaminated person, and a clean environment, such as the air which is breathed and filtered through a mask. The most efficient way to contain the spread of the virus is the use of face masks in public and indoor spaces. The efficiency of handmade masks can be less than 60% for silk masks for particles less than 300 nm, depending on the fabric, and a gap in masks can drastically reduce the filtration efficiency.^[1] Recently, a lot of work has focused on the development of masks to contain the spread of pathogen microorganisms, but few or none of them have focused on ventilation systems.^[2] Our research group has recently developed materials with bactericidal effects, such as electrospun nanofibers and cotton fabrics modified with silver nanoparticles.^[6,11]

In this context, the present work aimed to modify a commercial polyester filter media with copper nanoparticles to confer a bactericidal effect to the filter. The objective of this work was to synthesize copper nanoparticles and impregnate them to polyester commercial air conditioning filter media. It also tested an acid pretreatment on the filter prior to the CuNP impregnation to verify the effect of the adhesion of nanoparticles on the polyester fibre. The bactericidal effect of these modified filter media was tested against *Staphylococcus aureus* and *Escherichia coli* bacteria as gram-positive and gram-negative, respectively. The modification of filter media with copper nanoparticles aims to diminish the contamination of pathogen microorganisms in indoor rooms, and this technology can be used in several environments, such as hospitals, taxis, buses, airplanes, workplaces, and public spaces.

2 | MATERIALS AND METHOD

2.1 | Nanoparticles synthesis

Copper nanoparticles were synthesized by a redox reaction using polyvinylpyrrolidone (PVP) as a capping agent, following the Liu et al.^[12] method, with adaptations. The methodology consisted of first preparing 100 ml of a 0.2 mol/L $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ solution. In the same Erlenmeyer flask, 15 mmol/L of PVP was added. A 1.0 mol/L solution

of ascorbic acid in 40 ml of distilled water was prepared in another Erlenmeyer flask. Both flasks were submitted to an ultrasonication bath for 10 min to dissolve chemical reagents and minimize the effect of oxygen in reaction. The pH of both solutions was adjusted to 7 using NaOH to increase the pH solution, then solutions were submitted to heat (333 K), and solutions were mixed under agitation (70 rpm) and temperature for 4 h. The characteristic of the reduction reaction is the change in colour of the medium, which initially was blue due to the presence of Cu^{2+} ions and after the reaction changed to ochre, indicating the presence of copper nanoparticles.

The solution was maintained at 25°C for 12 h to precipitate nanoparticles and the supernatant was carefully discarded. The particles were centrifuged (DAIKI, 80-2B) at 4000 rpm for 10 min and washed with distilled water several times to remove all supernatant. Based on preliminary tests, nanoparticles were suspended in distilled water to obtain a 0.1% (w/w) solution.

2.2 | Filter media

The filter media used in this work are composed of polyester and are commercially sold for use in air conditioning systems, which were provided by FILTRACOM from Valinhos (São Paulo, Brazil). The manufacturer's specifications of the filter are shown in Table 1.

The commercial filter of the air conditioning system is composed of polyester and was cut in squares of 144 cm², following the impregnation process shown in the next section.

2.3 | Nanoparticles impregnation

The impregnation of synthesized nanoparticles was performed using a commercial bottle spray to deposit in filter media. In all extensions, 10 g of the 0.1% (w/w) CuNP suspension was sprayed through the surface of the filter. To remove all the excess water, the modified filters were submitted to an oven for 24 h at 50°C and stored in airtight bags before use. The final concentration of nanoparticles in the filter was 0.08 mg_{CuNP}/cm².

TABLE 1 Parameters of the polyester filter media

Composition	100% polyester
Colour	White
Thickness	5.5 ± 1.5 mm
Grammage	180 ± 10% g/m ²
Efficiency	80 ≤ ef ≤ 90 to particles above 0.4 μm

2.4 | Acid pretreatment with HCl

The pre-treatment in filter media previous to impregnation was done to improve the adhesion between fibre and nanoparticle. An HCl solution of 0.01 mol/L was prepared and the area of the filter was submerged into the solution for 5 min. The filter was removed with a tweezer and all the excess solution was removed before spraying the copper nanoparticles suspension (10 g in 144 cm² of filter). The CuNP solution was sprayed on the surface of the polyester fibre filter and the excess water was removed in an oven, as described in the previous section.

2.5 | X-ray diffraction (XRD)

Characterization of the nanoparticles by X-ray diffraction (Bucker XRD D8 Advance) was performed utilizing Cu K α radiation (1.5418 Å [0.154 18 nm]) in the scanning range of 2 θ between 20–70°. The crystallite size was measured from XRD patterns using the Scherrer equation:

$$FMHM(2\theta) = \frac{b \cdot \lambda}{D \cdot \cos\theta} \quad (1)$$

where FMHM (2 θ) is the line broadening at half the maximum intensity in radians; b is the Scherrer constant, related to the shape, which normally ranges between 0.89 = 0.94, and for nanoparticles its value is 0.9; λ is the X-ray wavelength ($\lambda = 0.154$ nm); D is the averaged dimension of crystallites in nanometres; and θ is the Bragg angle that corresponds to the position of the diffraction peak maximum, in rad.

2.6 | Scanning electron microscopy (SEM)

The arrangement and the morphology of the filter fibres, as well as the CuNPs impregnated to the fibres were analyzed with scanning electron microscopy (SEM) and the samples were previously covered with a thin layer of gold. The equipment used was an FEI Magellan 400. Energy dispersive X-ray spectroscopy (EDS) was also used to chemically characterize the sample.

2.7 | Antibacterial tests: Contact method

The agar-well diffusion method and the diffusion method were tested, but the high porosity of the filter media can bring inadequate results. To obtain results by the direct contact between impregnated filter media and bacterial

suspension and measure the bactericidal activity, the contact method^[11] was applied in this work.

Tryptone soya agar (TSA) was prepared according to the manufacturer's recommendation and distributed in Petri dishes, filling approximately one-third of the dishes' height. Petri dishes were maintained in the refrigerator until use. Gram-negative (*E. coli*, ATCC 8739) and gram-positive (*S. aureus*, ATCC 6535) bacteria were cultivated in tryptic soy broth (TSB) for 24 h. The cultivated bacteria suspension was collected by centrifugation (5000 rpm for 5 min) and the supernatant was discarded. The sediment was resuspended in phosphate buffer saline (PBS) and adjusted to 0.5 McFarland scale in a spectrophotometer at 600 nm wavelength and diluted to obtain a concentration of 10⁶ CFU/ml (6 logs).

Filters were cut in triplicate in equal pieces with 2.25 cm² and put in contact with the bacterial suspension (2 ml) for 4 h under agitation (160 rpm) and at room temperature. After the agitation period, aliquots of 100 μ l were diluted in 900 μ l of PBS to ensure that the inoculum was diluted 10⁵ times. In Petri dishes, 25 μ l of all concentrations of diluted inoculum were distributed and scattered with a Drigalski handle in the nutritive agar. The plates were incubated at 37°C for 24 h and the bacterial colonies were counted. A control (without any filter) and a blank (with the filter without nanoparticles) were simultaneously done to verify the filter effect in the colony of the bacterial suspension. All the samples were measured in triplicate. The reduction percentage was calculated according to Equation (2):

$$R(\%) = \left(\frac{C_o - C}{C_o} \right) \cdot 100 \quad (2)$$

where R is the bacterial reduction, in %; C_o is the initial bacterial concentration, in CFU/ml; and C is the final bacterial concentration, in CFU/ml.

3 | RESULTS AND DISCUSSION

3.1 | Synthesis and characterization of CuNP

The characteristic of the redox reaction is the colour of the suspension, which initially was blue and after 4 h turned to a brown/red colour, which indicates the presence of copper nanoparticles. The XRD patterns of particles demonstrated that the synthesized nanoparticles, when compared to the databank, are composed of a mixture of metallic copper (Cu⁰) and copper oxide (Cu₂O). Liu et al.^[12] (whose methodology was followed) also found the same compounds and suggested that Cu₂O is an intermediate product. The

reaction consists in transforming Cu^{2+} from CuSO_4 ions in $\text{Cu}(\text{OH})_2$ as a precursor when the pH of the solution was adjusted to 7 with NaOH. Then, $\text{Cu}(\text{OH})_2$ was reduced to Cu_2O , by ascorbic acid as redox agent, and finally Cu_2O was reduced to Cu^0 particles.^[12] The crystallite domain size was obtained by Equation (1), which relates the width of

the peak at half of its height with the size of the nanoparticle, using the XRD diffractogram peaks.^[13] The range of diffractogram from XRD analysis was in the range of $29\text{--}61^\circ$ (2θ). The size of the synthesized copper nanoparticles was measured by the Scherrer equation (Equation (1)) with a value of 37.88 ± 14.32 nm.

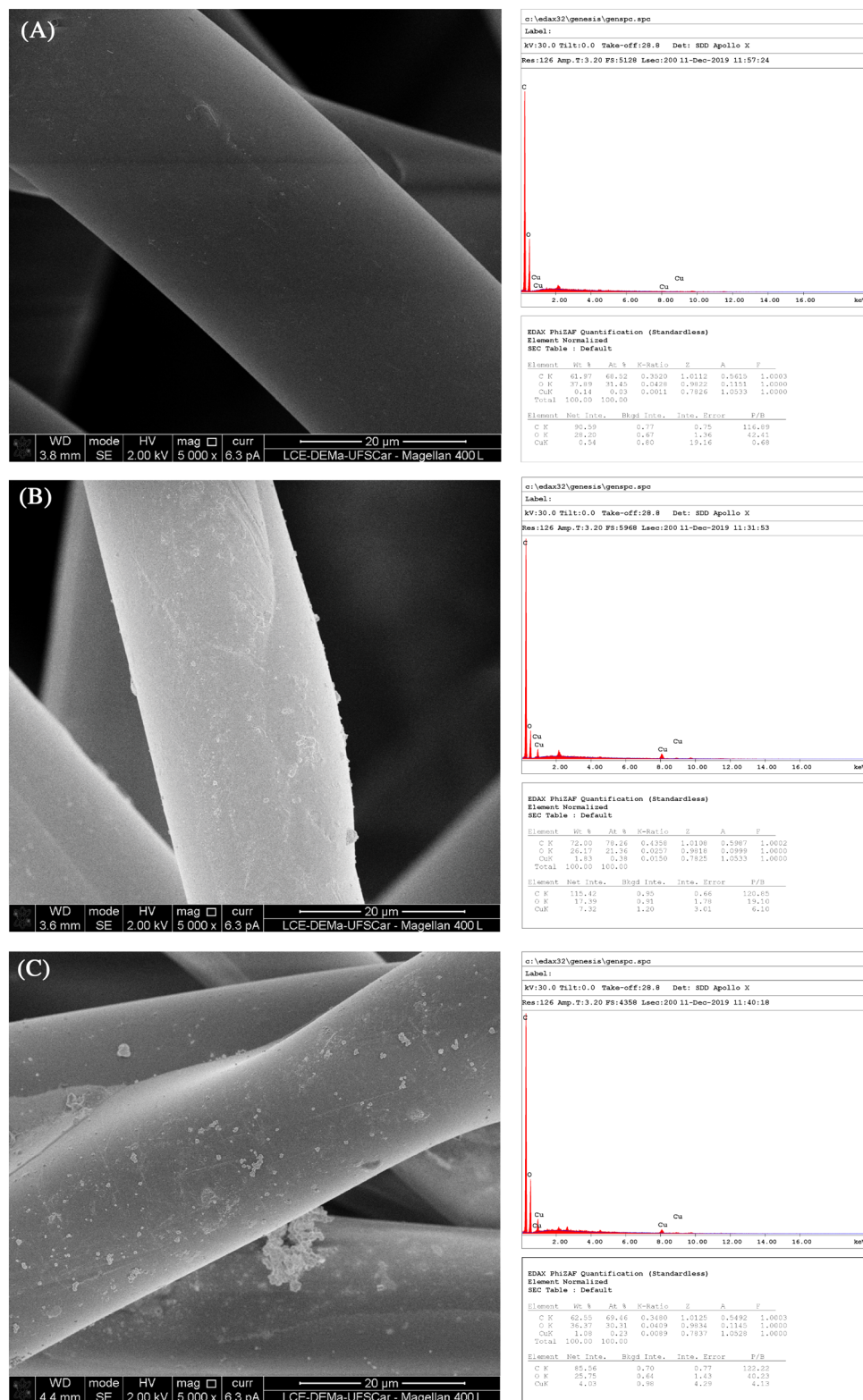


FIGURE 1 Scanning electron microscopy (SEM) images and energy dispersive X-ray spectroscopy (EDS) analysis of (A) blank filter, (B) impregnated with CuNP, and (C) impregnated with CuNP with HCl treatment. Yellow arrows indicate clusters of copper nanoparticles

3.2 | Filter modification

In this work, a low concentration of an HCl acid solution (0.01 mol/L) was used for a short time, to avoid any degradation in the fibre. Figure 1 shows the morphological structure of the fibre (blank and impregnated) followed by the EDS analysis of the area. We did not verify visual changes in the polyester fibre filter (Figure 1C).

The EDS analyses are useful to verify the chemical composition in a small area. Based on Figure 1, the equipment did not detect the presence of copper in blank fibre filter (Figure 1A), while for the impregnated with CuNP (Figure 1B) and impregnated with CuNP with HCl treatment (Figure 1C), EDS analysis confirmed the presence of the element copper, indicated in the peak at 8 KeV. In the EDS analysis shown in Figure 1C, the equipment also detected a peak at 2.6 KeV that corresponds to the peak of Cl (not indicated in Figure 1, present in Figure 1C) due to the chemical treatment. The SEM images were coupled to the EDS system and showed the presence of C, O, and Cu elements. The chemical structure of polyester $[C_{10}O_4H_8]_n$ evidences the presence of C and O in the fibre, and the other portion corresponds to the Cu_2O contribution. EDS analyses also evidenced that the nanoparticles impregnated to fibre filters were CuNP. A mean of 17 measurements in SEM in the equipment program showed an apparent diameter of 90.31 ± 46.42 nm. The difference between the size measurement in SEM and XRD suggests that the particles are polycrystalline.^[13]

3.3 | Antibacterial efficiency from the filter: Contact methodology

The agar-well diffusion test and the diffusion method were tested; however, we did not have good results,

probably due to the high porosity and thickness of the filter used. In contrast, the contact methodology from previous work by our research group^[6,11] showed good results and was able to measure the bacteria inhibition growth in the porous filter media. The colony growth of gram-positive and gram-negative bacteria are shown in Table 2, and in Figure 3 it is possible to verify the inhibition in log scale.

Results in Table 2 and Figure 2 show that the mixture of Cu and Cu_2O influenced the concentration reduction of the initial bacterial suspension (control). The evidence of the reduction due to the CuNP is shown when comparing the white filter with those impregnated with nanoparticles. For the filter impregnated just with copper, the reduction was 3 log (99.96%) to *S. aureus* and 4 logs (99.99%) for *E. coli*. When compared with the filter pretreated with acid, the reduction for *S. aureus* was more pronounced, at almost 4 logs, and at 3 logs for *E. coli*. The low difference between those values is commonly observed in analyses with microorganisms. We believe that the difference between those values is due to the technique, and not due to the chemical treatment. In Figure 2, it was observed that the pretreatment does not influence the bacterial growth for gram-positive bacteria, while for gram-negative bacteria the reduction was more pronounced when compared with initial suspension. The white filter was capable of reducing the bacterial suspension for gram-negative bacteria, likely indicating that polyester fibres can interact with the bacterial structure, inhibiting the growth.

The toxic mechanism of nanoparticles is studied but not completely understood, as the interaction between nanoparticles and bacteria can be very complex due to the size, shape, and composition of the nanoparticles.^[14] In this work, it was observed that bacterial reduction was more pronounced in gram-positive bacteria than in

TABLE 2 Bacteria count to gram-negative (*E. coli*) and gram-positive bacteria (*S. aureus*) and the % reduction in bacterial growth by the contact methodology for a commercial polyester filter media impregnated with CuNP and other with an acid pre-treatment

Sample	(CFU/ml)	R ₁ ^a (%)	R ₂ ^b (%)
<i>E. coli</i>			
Control bacteria	$6.8 \cdot 10^6 \pm 1.1 \cdot 10^6$	0	-
Blank	$2.6 \cdot 10^4 \pm 4.3 \cdot 10^4$	99.61	0
Impregnated (CuNP)	520 ± 396	99.99	98.02
Impregnated (CuNP) with pretreatment	$3.7 \cdot 10^3 \pm 1.1 \cdot 10^3$	99.95	86.04
<i>S. aureus</i>			
Control bacteria	$2.4 \cdot 10^6 \pm 8.2 \cdot 10^5$	0	-
Blank	$5.4 \cdot 10^5 \pm 1.6 \cdot 10^5$	77.37	0
Impregnated (CuNP)	826 ± 637	99.96	99.85
Impregnated (CuNP) with pretreatment	480 ± 435	99.98	99.91

^aBacterial reduction compared to control of bacteria culture.

^bBacterial reduction compared with the growth of the blank filter media in contact with bacterial suspension.

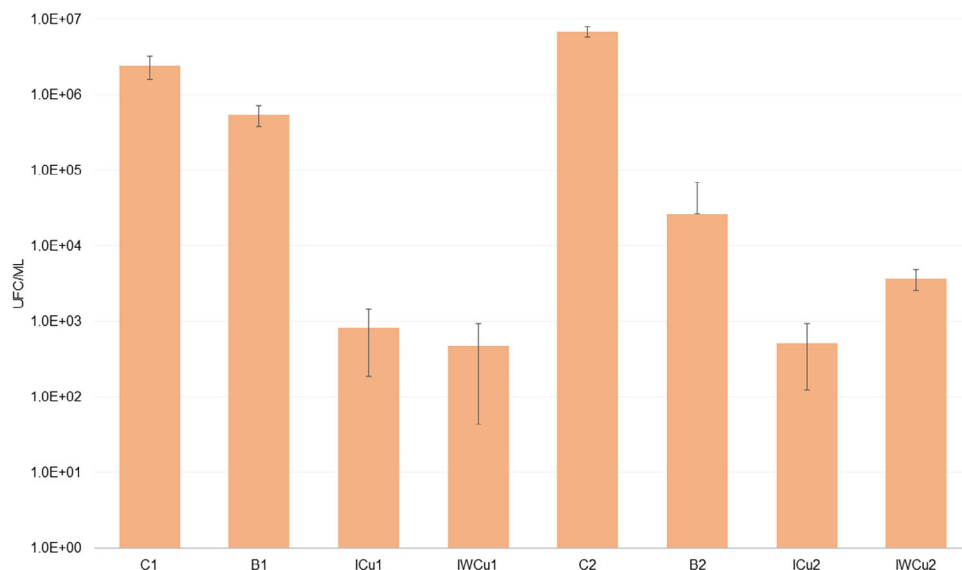


FIGURE 2 Counts in a logarithmic scale of gram-positive (*S. aureus*) and gram-negative (*E. coli*) showing the reduction in bacterial growth after contact methodology, for 4 h, between the suspension of control bacteria and the commercial filter impregnated with CuNP and also another one with an acid pretreatment. C—Bacterial suspension control; B—blank filter; ICu—impregnated with copper; IWCu—impregnated with copper with pretreatment acid (HCl). (1) Indicates *S. aureus* colonies and (2) indicates *E. coli* colonies

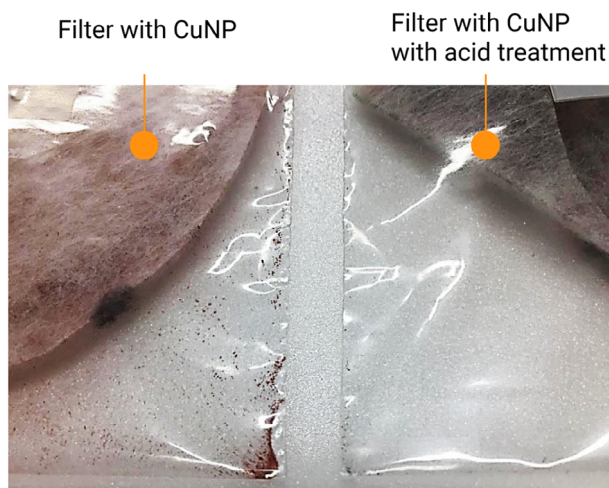


FIGURE 3 The visual aspect of polyester filter impregnated with copper nanoparticles

gram-negative bacteria. The interaction between the bacterial structure of the *E. coli* is lower due to the high resistance of this type of bacterium. The gram-negative bacteria have an outer membrane, an intermediate wall of peptidoglycan, and the plasma membrane, while gram-positive bacteria such as *S. aureus* have a cell wall of peptidoglycan followed by a plasma membrane. Hong et al.^[15] impregnated copper and silver in textiles, verifying that the effect of the nanoparticles in gram-negative bacteria was lower than in gram-positive bacteria. The same authors also added that the interaction between copper cations (Cu^+) and phosphorus groups found in the external membrane is high and leads to lower concentrations of those cations inside the structure. Also, the effect between Cu^+ interaction with gram-positive

bacteria is higher because it does not have an external wall, and it leads to a higher antimicrobial effect.

Other studies of the effect of copper nanoparticles to *E. coli*, *S. aureus*, and *Candida albicans* have observed that the effect was more pronounced in *E. coli*, reducing 99.9% with 2 h of contact for CuNP 5.3 nm in size,^[9] suggesting that the time, size and concentration are variables in the inhibition of microbes. The literature review suggested that bacterial inactivation occurs by the release of Cu^+ ions that interact with *E. coli* bacteria in the lipid bilayer, producing reactive oxygen species (ROS) and leading to lipid peroxidation and protein oxidation.^[9] Weaver et al.^[16] mentioned that the penetration of copper ions inside the cell is toxic, and that the interaction between bacteria and the same metal occurs due to the presence of an active surface and is vulnerable to redox reactions. Hashimoto et al.^[17] suggested that the presence of copper interrupts the respiratory chain, leading to cell apoptosis. All these mechanisms may occur simultaneously, inhibiting the growth of these microorganisms. The impregnation of CuNP in filters can reduce the growth of pathogens, as in the filters of ventilation systems, improving the air quality of indoor environments.

In this context, the particles impregnated to filter media are in the monovalent form of copper (Cu^+) due to the cuprous oxide (Cu_2O) and in the metallic form (Cu^0). Hashimoto et al.^[17] suggested that the monovalent form of ion copper is poorly explored when compared to the divalent form of the same material (CuO) that is widely explored. They developed micro and nanoparticles of these copper ions and studied the effect in virus inactivation, finding that in 30 min the divalent form of the ion (CuO) did not inactivate the influenza virus, while Cu_2O in the same period decreased the infection. This contribution

corroborates with other work^[8] that studied the effect of copper surfaces in a human coronavirus (229E) and verified that copper alloys inactivate the virus. They also suggest that the inactivation by the release of copper ions Cu^+ and the generation of ROS inside bacteria occurs in Cu^{+2} in a short time, while it occurs in the long term in Cu^{+1} . After this discussion, it is likely that the effect of copper nanoparticles is not limited to inactivating bacteria growth, but also may be involved in virus inactivation, including SARS-CoV-2; however, extra work is required for this.

Figure 3 shows the visual aspect of the filter media impregnated with copper nanoparticles.

Figure 3 evidenced the low attraction between the nanoparticle and the fibre filter of polyester. However, the chemical treatment positively influenced the adhesion force between fibres and copper nanoparticles, which visually do not release the nanoparticles. An acid treatment was also used by Jeyaraj et al.^[18] for 60 min in order to cleaning the material previous impregnation of natural dyes, indicating that the pretreatment can be also favourable to clean the fibre. In contrast, Nguyen and Trinh^[19] suggested that a concentrated alkaline of the CuNP synthesis results in mass loss of a PET filter. The same authors showed SEM images of a fibre filter before and after 120 min immersion in a 10 mol/L solution of NaOH and verified that the filter lost 5.2% in weight. However, the results shown in Figure 1C indicate that the pretreatment with a low concentration of acid (0.01 mol/L), for a short time (5 min) does not change the fibre filter visually, probably due to the mild conditions. It is also believed that the chemical treatment can modify the charge of the fibre and the nanoparticles can adhere more strongly, based on the interaction of van der Waals forces, than when the low concentrated chemical treatment is not applied. Supporting this argument, Cammarano et al.^[20] verified that polyester films after immersion in a NaOH solution increased the polarity of the films, changing the surface charge of the film. The increase of adhesion strength in the same work is also justified by the increase of OH groups in the film surface. Another possibility is that the HCl reacts with the structure of the polyester, creating active assets, and allowing a bond between fibre and nanoparticles. This inference is supported by the literature, where Bal and Behera^[21] suggest that a sulphuric acid at pH 1 for 48 h in contact with polyester fibre can break macromolecules and change the microstructure of the fibre, indicating that the acid solution can interact with the polyester structure and increasing the interaction between fibre-nanoparticle. The adhesion force and the interaction between fibre and nanoparticles are outside the scope of this work and further investigation should be conducted to explain this phenomenon, such as with atomic force

microscopy analysis. A review of the literature indicates that the chemical treatment increases the adhesion between fibre and nanoparticles, probably due to molecular interactions and increase of van der Waals interactions.^[22] It was verified through the colour change of the pretreated filter when compared to the filter impregnated with CuNP. This colour change probably occurs due to a chemical reaction between nanoparticles and the fibre, but this discussion should be further investigated. For now, it was observed that pretreatment was an option to avoid the release of nanoparticles maintaining high efficiency for pathogen bacteria. The chemical treatment approach in acid solution to surface modification is a simple method and does not require specialized equipment.

In the end, the application of copper nanoparticles to modify filters that can be used in the filtration system is an option for applications in, for example, portable air filtering, conferring a bactericidal effect to contain the spread of pathogen microorganisms in indoor rooms and prevention of nosocomial diseases. We also believe, after a review of the literature, that those nanoparticles could be effective for virus inactivation.^[8,23]

4 | CONCLUSIONS

A suspension of copper nanoparticles composed of metallic copper (Cu^0) and oxide copper (Cu_2O) was synthesized. The impregnation of those nanoparticles in commercial polyester filter media was done and a pretreatment prior to the impregnation of nanoparticles was tested. The CuNP impregnated to the polyester filter media reduced up to 99.99% of gram-negative bacteria and 99.98% of gram-positive bacteria. It was observed that the chemical pre-treatment with HCl also increased the adhesion between fibre and nanoparticles and maintained the bactericidal effectiveness. The modification of filter media with copper nanoparticles can be utilized in portable air filtering, in the air filtration of public transports, or even in hospital air filtration systems to prevent nosocomial diseases.

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PEER REVIEW

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ORCID

André Bernardo  <https://orcid.org/0000-0002-9345-3361>

REFERENCES

- [1] A. Konda, A. Prakash, G. A. Moss, M. Schmoltdt, G. D. Grant, S. Guha, *ACS Nano* **2020**, *14*, 6339.
- [2] F. J. Garcíá De Abajo, R. J. Hernández, I. Kaminer, A. Meyerhans, J. Rosell-Llompart, T. Sanchez-Elsner, *ACS Nano* **2020**, *14*, 7704.
- [3] L. Liao, W. Xiao, M. Zhao, X. Yu, H. Wang, Q. Wang, S. Chu, Y. Cui, *ACS Nano* **2020**, *14*, 6348.
- [4] C. D. Zangmeister, J. G. Radney, E. P. Vicenzi, J. L. Weaver, *ACS Nano* **2020**, *14*, 9188.
- [5] G. C. Tremiliosi, L. G. P. Simoes, D. T. Minozzi, I. Renato, D. C. B. Vilela, E. L. Durigon, R. Rahal, G. Machado, S. Carlos, bioRxiv preprint 2020, 152520, <https://www.biorxiv.org/content/10.1101/2020.06.26.152520v1> (accessed: June 29, 2021).
- [6] P. d. F. Rosa, M. L. Aguiar, A. Bernardo, *Water Air Soil Poll.* **2017**, *228*, 244.
- [7] M. Balcilar, Z. A. Ozdemir, *Physica A* **2019**, *534*, 122329.
- [8] S. L. Warnes, Z. R. Little, C. W. Keevil, *mBio* **2015**, *6*, e01697.
- [9] U. Bogdanovic, V. Lazi, V. Vodnik, M. Budimir, Z. Markovi, S. Dimitrijevi, *Mater. Lett.* **2014**, *128*, 75.
- [10] GBD 2017 Influenza Collaborators, *Lancet Resp. Med.* **2018**, *7*, 69.
- [11] A. C. C. Bortolassi, S. Nagarajan, B. de Araújo Lima, V. G. Guerra, M. L. Aguiar, V. Huon, L. Soussan, D. Cornu, P. Miele, M. Bechelany, *Materials Science and Engineering C* **2019**, *102*, 718.
- [12] Q. M. Liu, T. Yasunami, K. Kuruda, M. Okido, *T. Nonferr. Metal. Soc.* **2012**, *22*, 2198.
- [13] C. F. Holder, R. E. Schaak, *ACS Nano* **2019**, *13*, 7359.
- [14] A. Albanese, P. S. Tang, W. C. W. Chan, *Annu. Rev. Biomed. Eng.* **2012**, *14*, 1.
- [15] H. R. Hong, J. Kim, C. H. Park, *RSC Adv.* **2018**, *8*, 41782.
- [16] L. Weaver, J. O. Noyce, H. T. Michels, C. W. Keevil, *J. Appl. Microbiol.* **2010**, *109*, 2200.
- [17] K. Hashimoto, K. Sunada, M. Miyauchi, X. Qiu, Y. Kubota, H. Ishiguro, R. Nakano, J. Kajioka, Y. Yao (invs.), *US009572347B2*, 2017.
- [18] J. M. Jeyaraj, M. Arumugam, V. Kulandaiappan, *Revista Matéria* **2015**, *20*, 924.
- [19] V. T. Nguyen, K. S. Trinh, *Braz. J. Chem. Eng.* **2019**, *36*, 1553.
- [20] A. Cammarano, G. De Luca, E. Amendola, *Cent. Eur. J. Chem.* **2013**, *11*, 35.
- [21] S. Bal, R. C. Behera, *Journal of Minerals and Materials Characterization and Engineering* **2006**, *05*, 179.
- [22] C. Baley, F. Busnel, Y. Grohens, O. Sire, *Compos. Part A-Appl. S.* **2006**, *37*, 1626.
- [23] G. Borkow, S. S. Zhou, T. Page, J. Gabbay, *PLoS One* **2010**, *5*, e11295.

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