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SILVER (AGNPS) AND COPPER (CUSO4NPS) NANOFLUIDS, SYNTHESIS, CHARACTERIZATION AND THEM ANTIMICROBIAL PROPERTIES AGAINST PATHOGENIC MICROORGANISMS

Medina-Solano Andrea Marisol[1], Moreno-Rivera María de Lourdes[1], García-Ventura María Isabel[1], Balderas-López José Abraham[1] and Chávez-Sandoval Blanca Estela[2]*

1. Unidad Profesional Interdisciplinaria de Biotecnología, Department of Basic Sciences. Instituto Politécnico Nacional UPIBI-IPN. Av. Acueducto s/n, Barrio La Laguna Ticomán CP. 07340 G.A.M CDMX. México.
2. Escuela Nacional de Ciencias Biológicas. Department of Zoology. Instituto Politécnico Nacional ENCB-IPN. Prolongación de Carpio y Plan de Ayala s/n, Col. Santo Tomás C.P. 11340. Miguel Hidalgo CDMX. México.

Email : blanchavez29@gmail.com

ABSTRACT

The current "normality" derived from the COVID-19 pandemic has given rise to the need to use sanitizers that provide safety and efficacy.Nanomaterials like silver (AgNPs), cooper (CuSO4NPs), iron (FeNPs), ceramic (SiO2NPs), graphene carbon nanotubes, among others are used to develop and create multifunctional materials to fight the corona virus, because they have a broad spectrum of antimicrobial action. Nanofluids are not strong oxidants and are not expected to produce harmful disinfection by-products. Nanofluids exhibit good disinfection properties against a wide range of bacteria, including Gram-negative, Gram-positive and bacteria spores, viruses, yeasts, and fungi. Several patents divulge the typically used types of nanofluids and their possible disinfection/ decontamination mechanisms. Currently, a great variety of cleaning articles are added with nanomaterials, mostly medical supplies, such as face masks or mouth covers, among others.In this work, the effectiveness of silver and copper nanofluids was being evaluated in *Pseudomonas sp.*, *Escherichia coli*, *Staphylococcus aureus* and *Salmonella sp.* strains. Those strains were chosen for their medical importance.

***Keywords*:** Nanofluids, AgNPs, $CuSO_4$ NPs, Sanitizer, Microbiological evaluation

INTRODUCTION

Nanomaterials display unique and well-defined physical and chemical properties, intrinsic to their size, making them useful for biomedical applications, such as: very high surface area to volume ratio, biocompatibility, biodegradation, safety for human ingestion, capacity to support surface modification and therefore, to be combined with other bioactive molecules or substrata and more importantly being seemingly not attracting antimicrobial resistance (Grumezescu., *et al* 2014; Abram *et al*., 2018; Chavez-Sandoval *et al*., 2020; Chavez-Sandoval *et al*., 2021; Loredo-Becerra *et al*., 2022). Thus, there is a boom in their use in various fields and, recently, due to the coronavirus pandemic fighting.

The chemical composition of many sanitizers makes them harmful or toxic not only to humans, but also to other organisms, in addition, its spectrum of action, activation start time, activity time, residual effect, toxicity, penetration capacity and possible materials or circumstances that inactivate them may vary from one product to another (Ramaiah *et al*., 2021; Phakatkar *et al.*, 2023).

The most widely types of nosocomial infections are catheter-related bloodstream infection, ventilator-associated pneumonia, surgical site infection and catheter-associated urinary tract infection, which are often caused by *Staphylococci*, *Pseudomonas* and *Escherichia coli*, among others.

To reduce the frequency of such infections and substantially decrease morbidity and mortality, postoperative antibiotic therapy was the first-choice method to fight against these infections. Nevertheless, problems associated with their long-term use began to appear.

The major problem is antimicrobial resistance, especially the acquired type. Of greatest concern are methicillin- and vancomycin-resistant *Staphylococcus aureus*, vancomycin-resistant *Enterococcus*, and extended-spectrum -lactamase-producing Gram-negative bacilli. Due to these disadvantages of conventional antibiotics, new methods of prevention are being researched (Polívková *et al*., 2017).

In this work we synthesize and characterize metal nanoparticles (AgNPs and CuSO4NPs) and evaluate their effectiveness against other disinfectants and their antimicrobial properties against pathogenic microorganisms (*Pseudomonas sp*., *Escherichia coli*, *Staphylococcus aureus* and *Salmonella sp.*).

MATERIALS AND METHODS

The materials and equipment used are in the Interdisciplinary Professional Unit of Biotechnology (UPIBI) and the nanoparticles obtained were characterized by TEM in the Center for Nanoscience and Micro and Nanotechnologies of the IPN.

All reagents used in the synthesis of nanoparticles were analytical grade.

For AgNPs we use:
Sodium Citrate ($Na_3C_6H_5O_7$) 0.5 g
Silver Nitrate (AgNO3) 0.7 g
Double-distilled water

For CUSO4NPs we use:
Ascorbic acid (C6H8O6) 1 g
Copper sulphate (CuSO4) 4 g
Double-distilled water

The following strains of bacteria were selected because of their medical significance: *Escherichia coli, Staphylococcus aureus, Pseudomonas and Salmonella sp.*
The bacterial strains were obtained from the UPIBI culture collection.

Nanoparticles Synthesis

Considering that this work was carried out during the COVID 19 pandemic, we decided to synthesize the nanoparticles using microwaves, a 700-watt *Midea* microwave oven, model MMDP07S2BW, was used.

The synthesis method used is as described in Chavez-Sandoval et al., 2016, with the innovation that we use a microwave instead of traditional synthesis. The samples were labeled and stored at 4°C to be later characterized.

Nanoparticles UV-Vis Characterization

For this characterization, a GBC UV-Vis spectrophotometer, model Cintra 1010, with a wavelength range of 190 to 1100 nm was used.

Nanoparticles TEM Characterization

The equipment used was a scanning electron microscope in transmission mode of the JEOL brand model JEM-ARM200F (Japan) of the Electron Microscopy Laboratory of the Center for Nanosciences and Micro and Nanotechnologies (CNMN) of the IPN.

Evaluation of the effectiveness and antimicrobial activity of synthesized nanoparticles (AgNPs and $CuSO_4$NPs).

The antimicrobial susceptibility test was performed using the technique Kirby-Bauer Disk Diffusion Susceptibility Test, (Yang et al., 2019), this antimicrobial susceptibility test was used to observe the inhibition halo presented by copper nanoparticles ($CuSO_4$NPs) and silver nanoparticles (AgNPs) synthesized with respect to each selected strain and whit 2 trade-mark sanitizers.
The interpretation of the results of Kirby-Bauer Disk Diffusion Susceptibility Test, is based on the measurement of the diameter of the inhibition zone. The larger the diameter of the area, the greater the susceptibility of the bacterial strain to the antimicrobial agent. Resistant bacterial strains will have smaller or no zones of inhibition.

Use of nanostructured sanitizer

The Sanitizer developed in this work can be used by spraying, misting or fine spraying by means of a spraying device, graduating the droplet size from 50 to 200 μm. In this way, the sanitizer acts mainly in the liquid phase by moistening the surfaces, however, another advantage is that in a small proportion it also does so in the gas phase.

It can also be used by immersion by simply immersing the object in the solution for a minimum contact time of 5 to 10 minutes.

It can be used on any surface, floors, utensils, locker rooms, people, leathers, skins, medical supplies, among others.

RESULTS AND DISCUSSION

Synthesis

Silver nanoparticles Ag NPs of approximately 20 nm and copper $CuSO_4$ NPs of approximately 5-100 nm were obtained by the chemical reduction method using microwaves (Fig.1).

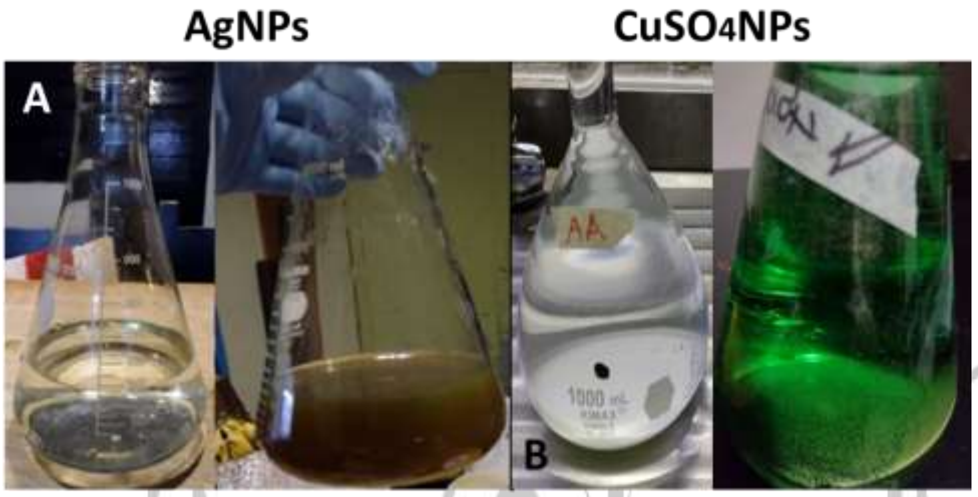

Figure 1. A) AgNPs before and after microwaving the reagents. B) CuSO4NPs before and after microwaving the reagents

The synthesis methods using microwaves allowed the obtaining of silver AgNPs and copper CuSO4NPs nanoparticles.

UV-Vis Characterization

According to the literature, a peak at 400 nm corresponds to silver AgNPs nanoparticles less than 5 nm in diameter, whereas, if the absorption peak is shifted to 420 nm, the predominant size of AgNPs is around 20 nm (Haiss et al., 2007).

Based on the above, the fact that the peak absorption of our nanoparticles was 425 nm is an indication that they have a size of around 20 nm.

On the other hand, copper nanoparticles had a peak of 575 nm, so their size ranges from 5 to 100 nm (Fig. 2).

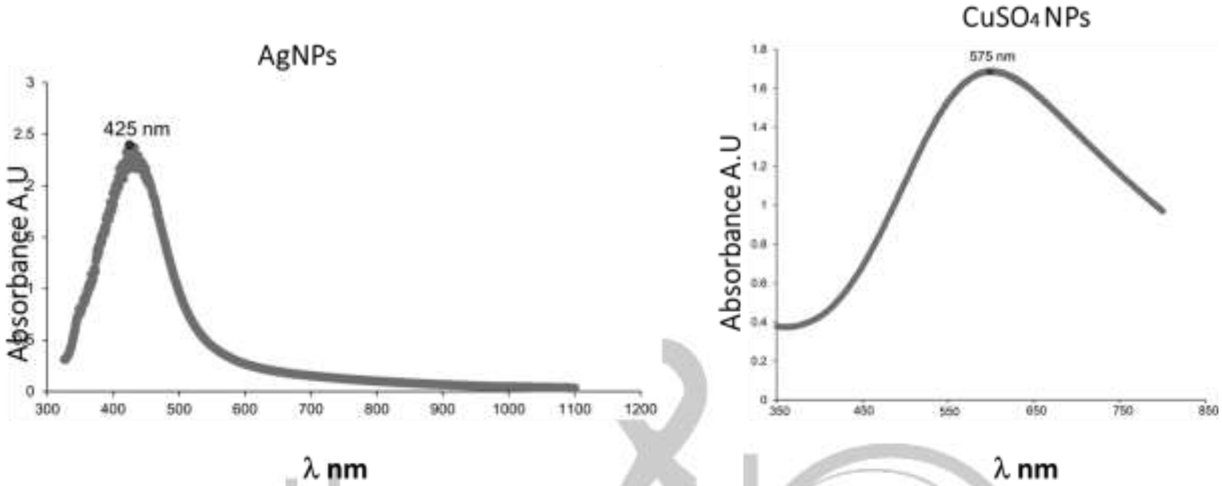

Figure 2. UV-Vis Characterization of AgNPs and CuSo4 NPs

TEM Characterization

Figures 3 and 4 shows the images obtained by Transmission Electron Microscopy (TEM). This characterization confirms that AgNPs sizes are approximately 20 nm. However, 5 nm nanoparticles are also observed, so we have variable shapes and sizes, spherical mostly.

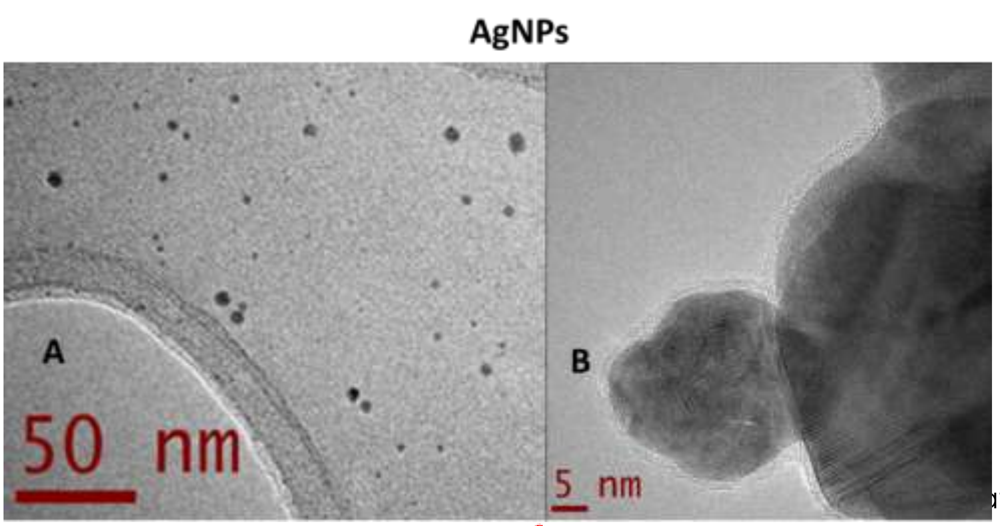

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Figure 3. AgNPs A. 50 nm scale. B. 5 nm scale

$CuSO_4$NPs are observed aggregated (Fig. 4), perhaps because the characterization was performed approximately 3 months after synthesis as we had to wait for this analysis, or perhaps because of the reactants themselves. CuSO4NPs. Therefore, with this characterization, we could not observe the size or shape.

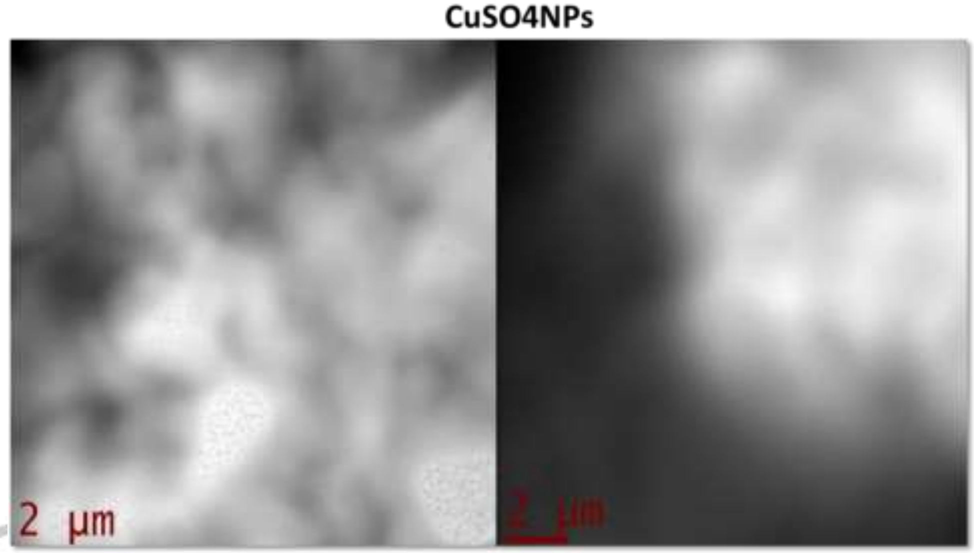

Figure 4. CuSO4NPs.A and B are at a scale of 2 µm

In addition, nanoparticles have a natural tendency to form agglomerates or aggregates.

Nanofluid formulation

Our formula includes surfactants, silver or copper nanoparticles and double-distilled water, optionally essences can be added.

It is not recommended that it be mixed with chlorine or alcohol as these chemicals can cause the nanoparticles to precipitate and lose their effectiveness.

Antimicrobial effect by Kirby-Bauer Disk Diffusion Susceptibility Test

The bacterial strains evaluated showed growth and 24 hours after incubation, the results were read.

The diameter of the zone was measured including the 7 mm diameter of the disc, the results for each trial were reported in Figure 5-6 and Tables 1-2.

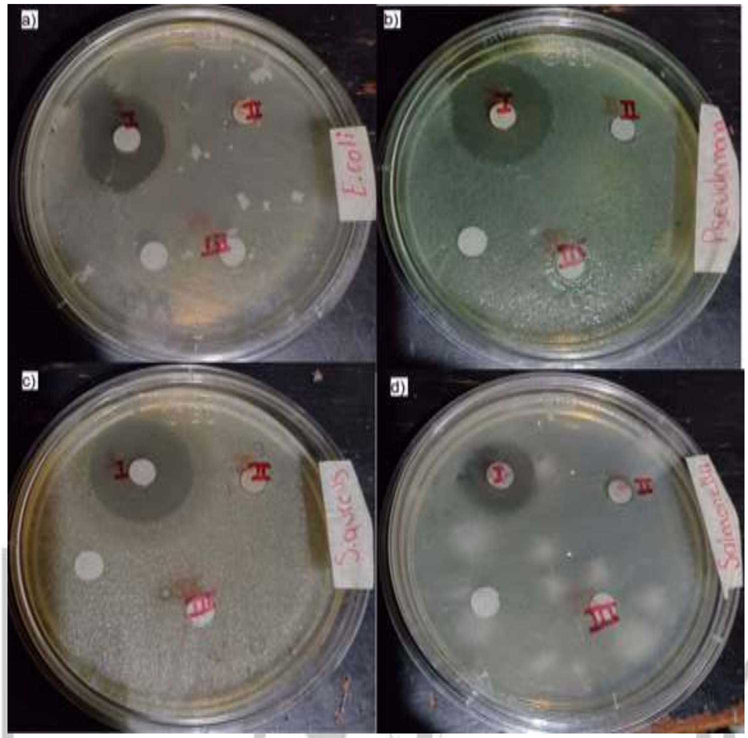

Figure 5. Inhibition zones generated by the copper nanoparticles CuSO4NPs (I), AgNPs (II) and the **Trademark 1** (III), against the bacterial strains a) *Escherichia coli*, b*) Pseudomona sp.* c) *Staphylococcus aureus*, and d) *Salmonella sp*. Images obtained 24 hours after incubation

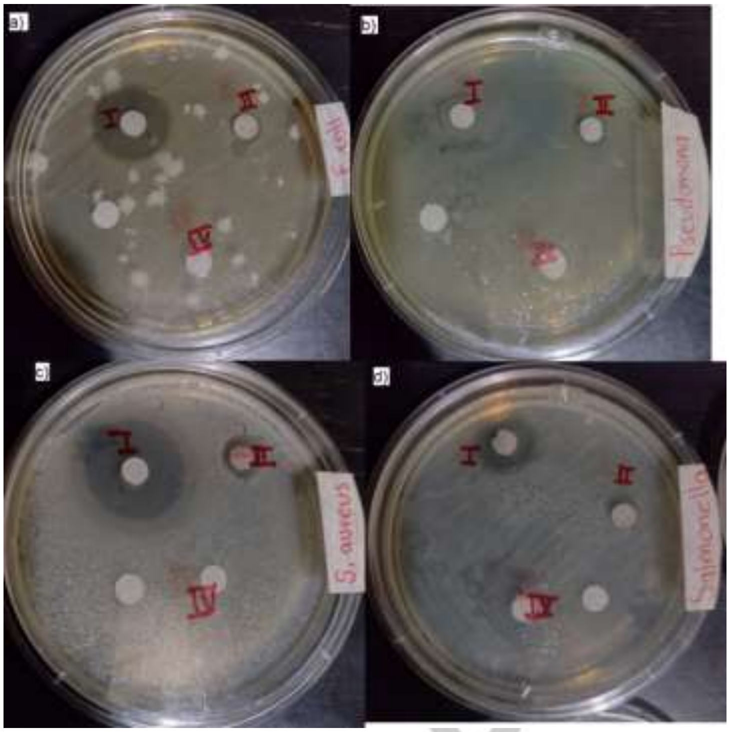

Figure 6. Inhibition zones generated by the copper nanoparticles CuSO4NPs (I), AgNPs (II) and the **Trademark 2** (IV), against the bacterial strains a) *Escherichia coli*, b*) Pseudomona sp.* c) *Staphylococcus aureus*, and d) *Salmonella sp*. Images obtained 24 hours after incubation.

Table 1. Results of Kirby-Bauer Disk Diffusion Susceptibility Test.

Microorganisms	Kirby-Bauer Disk Diffusion Susceptibility Test in mm. 24 hours after incubation.			
	$CuSO_4NPs$	AgNPs	Trade-mark 1	Trade-mark 2
Escherichia coli	22	10	10	10
Pseudomona sp.	20	9	9	9
Staphylococcus aureus	26	10	8	9
Salmonella sp.	17	9	8	8

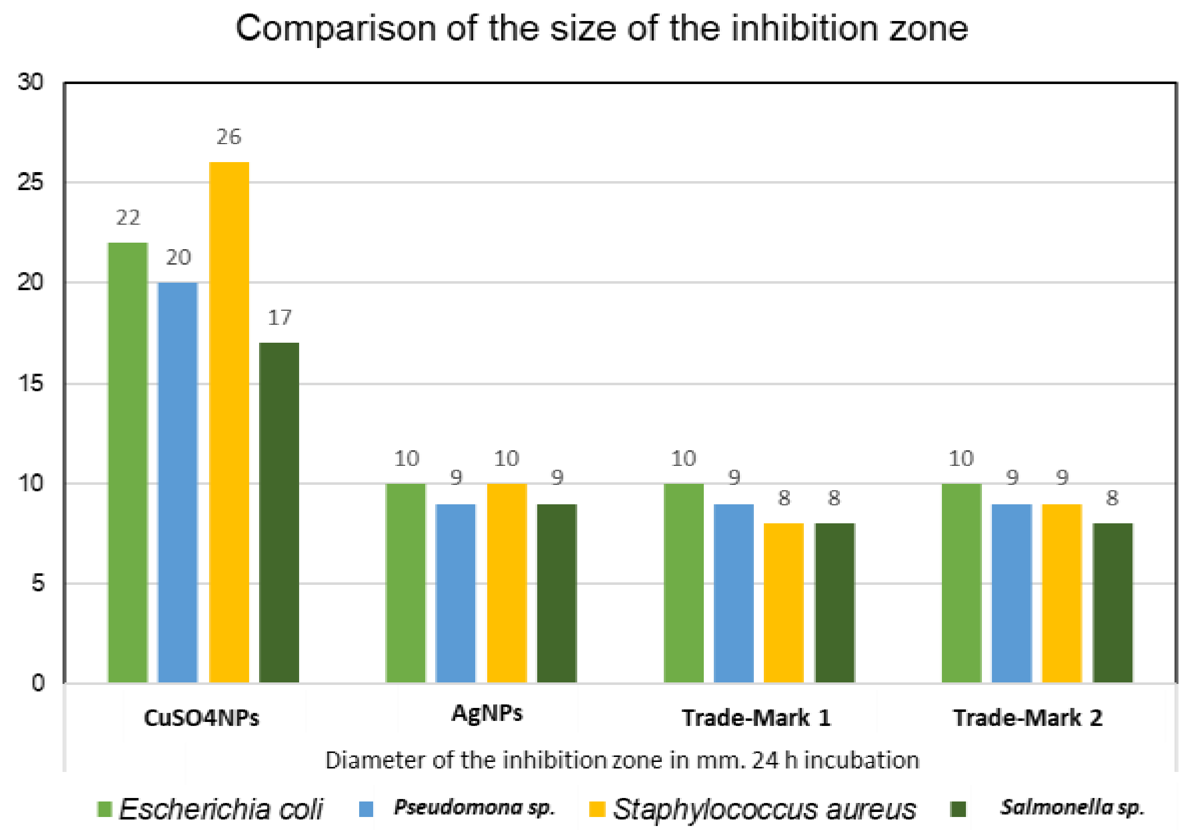

Figure 7. Diameter of the inhibition zone in mm. After 24 h incubation

Based on the results obtained, a better antimicrobial effect of copper nanoparticles was observed compared to silver nanoparticles, since copper nanoparticles presented larger inhibition diameters in all assays. However, toxicity must be considered.

The mechanisms of antibacterial action of nanoparticles are not yet well elucidated, so although this mechanism of action against bacteria is not yet fully defined, some authors mention the possible effects of nanoparticles on the bacterial cell. The ions released by the nanoparticles bind to the cell wall and break it apart. These ions inside bacterial cells disrupt biochemical processes since these ions can generate ROS (reactive oxygen species) that interacts with membrane proteins; affecting its permeability and inclusively can affect DNA (Antonoglou *et al*., 2017; Loredo-Becerra *et al.*, 2022; Paesa *et al.*, 2023).

CONCLUSIONS

Silver nanoparticles AgNPs and copper $CuSO_4$ NPs were obtained by the chemical reduction method using microwaves.

The UV-Vis characterization showed that silver nanoparticles AgNPs and copper CuSO4NPs were obtained since the spectra obtained presented the surface resonance plasmon, a physical property that is only exhibited by metallic materials when they have nanometric dimensions, which in the case of silver nanoparticles was 425 nm and for copper nanoparticles it was 575 nm.

TEM characterization corroborated the presence of mostly spherical silver nanoparticles (AgNPs) of approximately 20 nm. However, for copper nanoparticles (CuSO4NPs), their size and morphology could not be corroborated.

Regarding the evaluation of the antimicrobial activity of the synthesized nanoparticles, it is concluded that both copper nanoparticles ($CuSO_4$NPs) and silver nanoparticles (AgNPs) showed antimicrobial activity against the evaluated strains (*Escherichia coli, Staphylococcus aureus, Pseudomona sp. and Salmonella*).

However, the copper nanoparticle sanitizer turned out to be more effective, since in the susceptibility tests there were inhibition halos 75% greater than those presented with the sanitizer made with AgNPs.

These results allow us to conclude that the nanostructured sanitizers we formulated from AgNPs and $CuSO_4$NPs obtained by chemical reduction by microwaves have a potential use as disinfectant agents against *Escherichia coli, Staphylococcus aureus, Pseudomonas and Salmonella.*

PERSPECTIVES

An important question that needs to be studied is how nanoparticles act against pathogenic microorganisms, so we will carry out experiments involving different sizes of nanoparticles as well as different pathogenic microorganisms.

Although the reagents used in this work are not harmful or toxic, we recommend using nanoparticles obtained using green chemistry to avoid allergies to users as well as damage to the environment.

Another important question is the commercialization of nanostructured sanitizers. Those already on the market do not indicate which nanoparticles they are, what size or their composition. For this reason, we also propose to carry out a study that indicates the percentage of the population that already uses them as well as their experience.

Conflict of interest statement

The author declares no conflict of interest.

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