Chapter 10 Health Area

SILVER AND COPPER NANOFLUIDS, SYNTHESIS, CHARACTERIZATION AND THEIR ANTIMICROBIAL PROPERTIES AGAINST PATHOGENIC MICROORGANISMS

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Abstract

Nanofluids, which are not strong oxidants and are not expected to produce harmful disinfection by-products, have demonstrated excellent antimicrobial properties against a range of microorganisms, including Gram-negative and Gram-positive bacteria, viruses, yeasts, and fungi.

Given the rise in antimicrobial resistance and the limitations of traditional antibiotics, there is an urgent need for new disinfection alternatives. Several patents reveal the commonly used types of nanofluids and their potential disinfection and decontamination mechanisms. This study focuses on the synthesis and characterization of silver and copper nanofluids, evaluating their effectiveness against pathogenic strains such as *Pseudomonas sp., Escherichia coli, Staphylococcus aureus,* and *Salmonella sp.*

These bacterial strains were chosen due to their medical importance and role in common nosocomial infections. The World Health Organization (WHO) identifies *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Salmonella sp.* as critical pathogens because of their high resistance to multiple antibiotics and their involvement in severe infections.

The synthesized nanofluids demonstrated significant antimicrobial activity. These findings suggest that nanostructured sanitizers could be a viable alternative to traditional disinfectants, potentially reducing infection rates and combating antimicrobial resistance.

Keywords: Pathogenic microorganisms, Antimicrobial resistance, Nosocomial infections, metallic nanoparticles, nanofluids

1. Introduction

The significant health implications of pathogenic microorganisms have driven extensive research into new antimicrobial strategies. Metallic nanoparticles, specifically silver (AgNPs) and copper ($CuSO_4$NPs), are gaining considerable attention due to their unique physicochemical properties and potent biological activities. These nanomaterials offer a high surface area to volume ratio, biocompatibility, and the ability to support surface modifications, making them ideal candidates for medical applications. Crucially, they do not promote antimicrobial resistance, a major drawback associated with conventional antibiotics [1].

Metallic nanoparticles have attracted considerable attention in the realms of physics and chemistry because of their unique characteristics compared to bulk materials. They are utilized in drug delivery systems, biomolecules, antimicrobials, nucleic acids, and play crucial roles in diagnostics and treatment.

To further expand on the significance of innovative antimicrobial strategies, it's crucial to acknowledge the limitations and risks associated with conventional sanitizers and antibiotic therapies. 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 [2]. Moreover, nosocomial infections, such as catheter-related bloodstream infections, ventilator-associated pneumonia, surgical site infections, and catheter-associated urinary tract infections, predominantly caused by *Staphylococcus*, *Pseudomonas*, and *Escherichia coli*, pose significant health risks [3].

Addressing these challenges requires new methods of prevention and treatment. Postoperative antibiotic therapy, while initially effective, has led to increased antimicrobial resistance, especially among methicillin- and vancomycin-resistant *Staphylococcus aureus*, vancomycin-resistant *Enterococcus*, and extended-spectrum beta-lactamase-producing Gram-negative bacilli. This scenario necessitates the exploration of alternative antimicrobial approaches [3].

In response to these challenges, this study focuses on synthesizing and characterizing silver and copper nanofluids (AgNPs and $CuSO_4$NPs) and evaluating their antimicrobial properties against medically significant pathogenic microorganisms: *Pseudomonas sp.*, *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella sp.* Through this inquiry, leveraging the unique properties of these nanofluids aims to develop effective and safe disinfectants applicable in various healthcare settings.

2. Metodology

2.1. *Materials and methods*

The materials and equipment utilized for this study were sourced from the Pharmaceutical Microbiology Laboratory at UPIBI. All reagents employed in the nanoparticle synthesis and evaluation of antimicrobial activity were of analytical grade.

The bacterial strains evaluated were *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853), and *Salmonella* (ATCC 14028).

The analyses for TEM (Transmission Electron Microscopy) characterization were conducted at the Center for Nanosciences and Micro/Nanotechnologies (CNMN) of IPN.

2.2. *Nanoparticles Synthesis*

Nanoparticles were synthesized using the Turkevich method, which involves the use of microwaves instead of traditional synthesis, as described in Turkevich et al., 1951. This approach represents an innovative deviation from conventional methods.

The reagents used were: Silver Nitrate ($AgNO_3$) (PM ≈ 169.87 g/mol) ≥ 99.0%, Sodium Citrate ($Na_3C_6H_5O_7$) (PM ≈ 294.10 g/mol) ≥ 99.0%, Ascorbic Acid ($C_6H_8O_6$) (PM ≈ 176.13 g/mol) ≥ 99.0%, Copper(II) Sulfate Pentahydrate ($CuSO_4 \cdot 5H_2O$) (PM ≈ 2 49.685 g/mol) ≥ 99.0%, distilled water, and double-deionized water (Milli-Q). All these reagents were purchased from Sigma-Aldrich.

The samples were labeled and stored at 4°C to be later characterized.

3. 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 employed. UV-Vis spectroscopy is a common technique used to characterize nanoparticles based on their absorption of ultraviolet and visible light.

UV-Vis characterization provides valuable information about the optical properties of the synthesized nanoparticles, confirming their successful synthesis and providing initial insights into their size and morphology. Furthermore, the presence of absorption peaks is related to the phenomenon of surface plasmon resonance, a unique physical property exhibited by metallic materials when they have nanoscale dimensions.

3.1. *Nanoparticles TEM Characterization*

Transmission Electron Microscopy (TEM) was employed for 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.

The objective of employing this technique was to obtain high-resolution images of the synthesized nanoparticles, allowing for precise characterization of their morphology, size distribution, and structural properties.

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

The antimicrobial susceptibility test was performed using the Kirby-Bauer Disk Diffusion Susceptibility Test [4]. 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 with two trademark sanitizers. The objective of using this technique is to determine the antibacterial efficacy of the synthesized nanoparticles by measuring the zones of inhibition they produce against various bacterial strains.

The interpretation of the results of the 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.

3.3. *Use of Nanostructured Sanitizer*

The sanitizer developed in this work offers versatile application methods. It can be used by spraying, misting, or fine spraying using a spraying device, with the ability to adjust the droplet size from 50 to 200 µm. This allows for effective coverage of surfaces, as the sanitizer primarily acts in the liquid phase by moistening the surfaces. Additionally, a small proportion of the sanitizer also acts in the gas phase, enhancing its efficacy.

Alternatively, the sanitizer can be used by immersion, where objects are simply submerged in the solution for a minimum contact time of 5 to 10 minutes. This method ensures thorough disinfection of items.

The nanostructured sanitizer is suitable for use on various surfaces, including floors, utensils, locker rooms, human skin, leather, and medical supplies, among others. Its broad applicability makes it a valuable tool for maintaining hygiene and preventing the spread of pathogens.

4. Results and discussion

4.1. *Synthesis*

The application of the chemical reduction method with microwaves resulted in the production of silver nanoparticles (AgNPs) with an estimated size of around 20 nm and copper nanoparticles ($CuSO_4$NPs) ranging between 5 to 100 nm. The use of microwave synthesis techniques facilitated the generation of both silver and copper nanoparticles, as depicted in Figure 1.

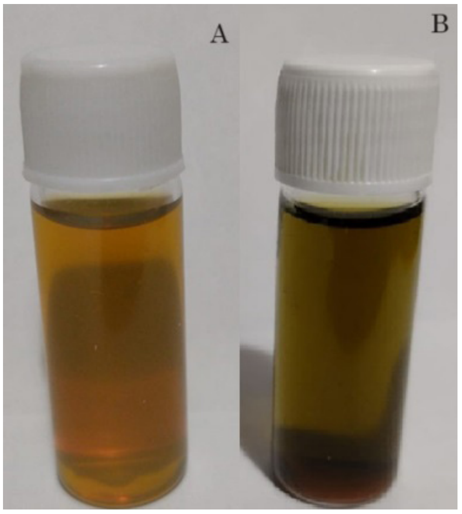

Figure 1. A) Suspensions of silver nanoparticles (AgNPs) and B) copper nanoparticles ($CuSO_4$NPs).

4.2. *UV-Vis Characterization*

UV-Vis spectroscopy was conducted to obtain absorption spectra of the nanoparticle suspensions and observe the phenomenon known as the "surface plasmon resonance (SPR)," which corresponds to a physical property exhibited only by metallic materials when they have nanoscale dimensions. This appears as a characteristic strong absorption band. Therefore, these spectra confirm the presence of nanoparticles and provide an estimate of their approximate size.

Characterization through UV-Vis spectroscopy revealed distinct peaks, indicating the presence of silver and copper nanoparticles. The absorption peak observed for AgNPs at 425 nm suggested a predominant size of approximately 20 nm, consistent with previous literature [5]. On the other hand, copper nanoparticles displayed a peak at 575 nm, suggesting a size range spanning from 5 to 100 nm (Figure 2).

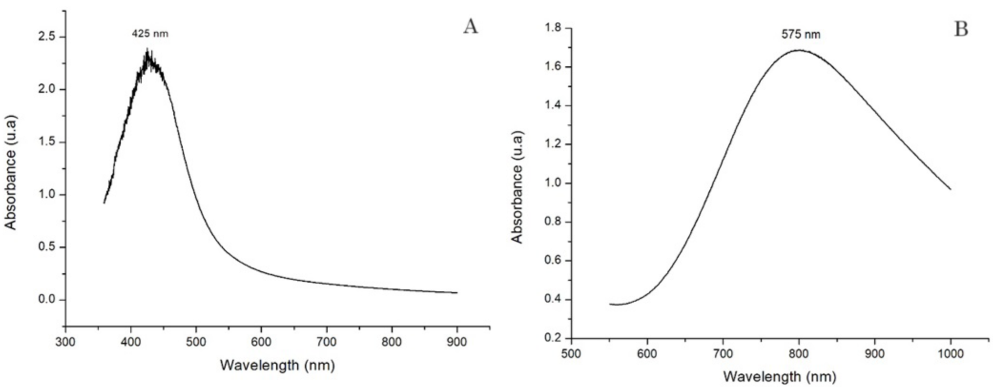

Figure 2. UV-Vis spectrum of silver nanoparticles (AgNPs) and copper nanoparticles ($CuSO_4$NPs).

4.3. *TEM*

The TEM analysis presented in Figures 3 provides insights into the structural characteristics of silver nanoparticles. Specifically, the images confirm the presence of AgNPs with an average size of around 20 nm, alongside smaller 5 nm particles, indicating variability in size and shape, predominantly spherical. Conversely, $CuSO_4$NPs appear aggregated, potentially influenced by the timing of the characterization process, conducted approximately three months post-synthesis, or inherent properties of the reactants. This aggregation precluded a clear observation of the size and shape of $CuSO_4$NPs during characterization.

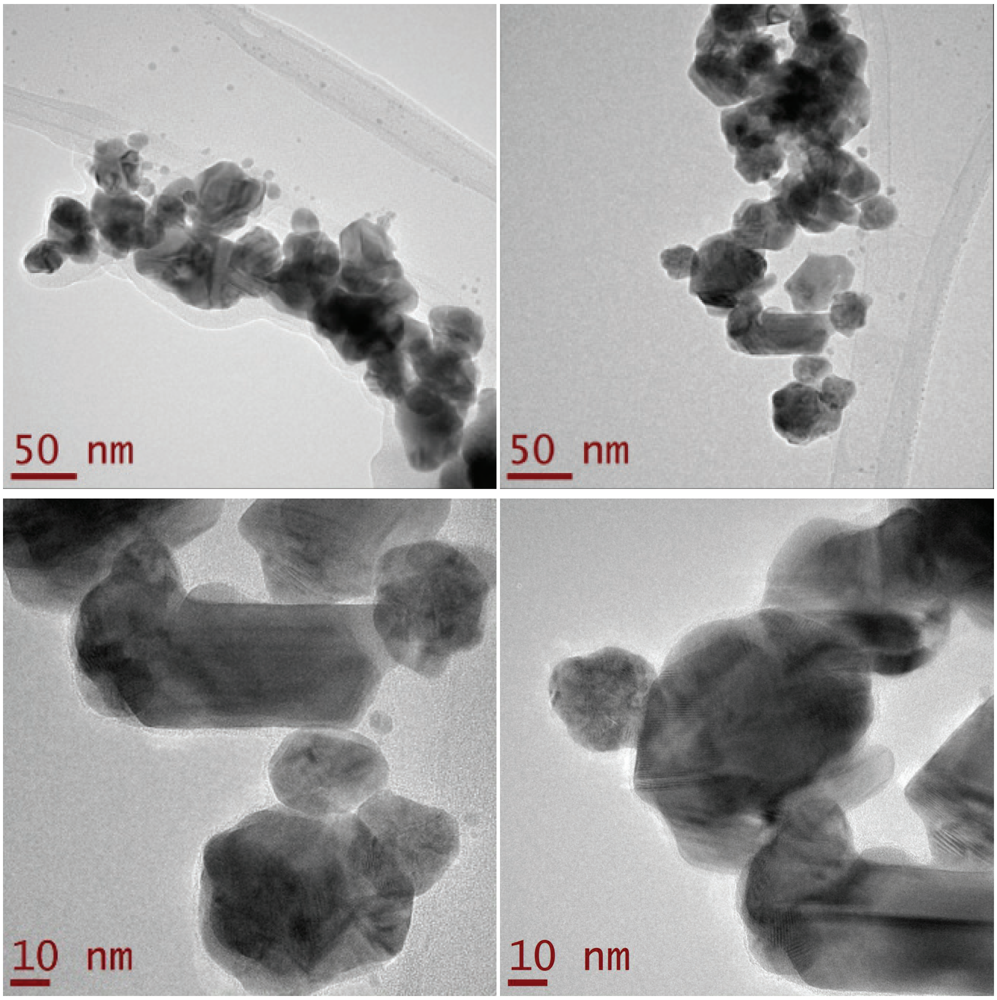

Figure 3. Silver nanoparticles (AgNPs).

4.4. *Antimicrobial effect by Kirby-Bauer Disk Diffusion Susceptibility Test*

The antimicrobial efficacy of the synthesized nanoparticles was assessed using the Kirby-Bauer Disk Diffusion Susceptibility Test. Following incubation for 24 hours, bacterial growth was observed, and the resulting inhibition zones were measured, including the 7 mm diameter of the disc. Detailed results for each trial were documented in Figures 4-5 and Tables 1, providing a comprehensive overview of the antimicrobial activity exhibited by the nanoparticles against the evaluated bacterial strains.

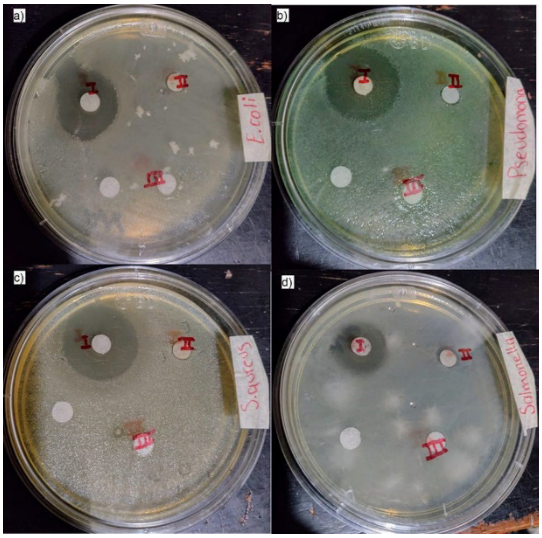

Figure 4. Inhibition zones generated by the copper nanoparticles CuSO4NPs (I), AgNPs (II) and the Trademark 1 (III), against the bacterial strains a) *Escherichia coli*, b) *Pseudomonas sp.* c) *Staphylococcus aureus*, and d) *Salmonella sp.* Images obtained 24 hours after incubation.

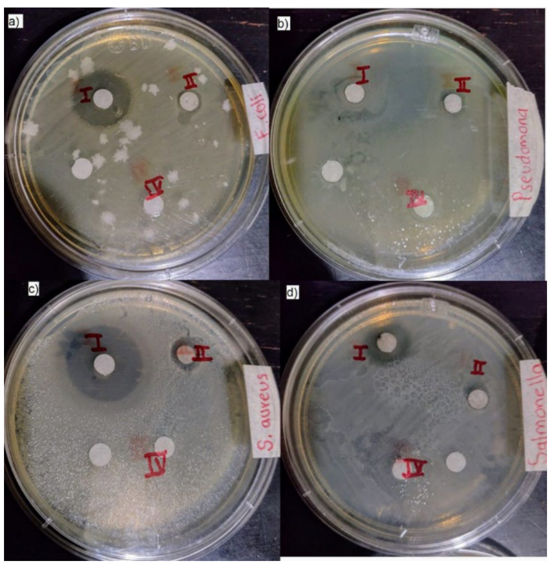

Figure 5. Inhibition zones generated by the copper nanoparticles $CuSO_4$NPs (I), AgNPs (II) and the Trademark 2 (IV), against the bacterial strains a) *Escherichia coli*, b) *Pseudomonas sp.* c) *Staphylococcus aureus*, and d) *Salmonella sp.* Images obtained 24 hours after incubation.

Microorganisms	Kirby-Bauer Disk Diffusion Susceptibility Test (mm) 24 hours after incubation			
	I. CuNP's	II. AgNP's	III. Trade-Mark 1	IV. Trade-Mark 2
Escherichia coli	22	10	10	10
Pseudomonas	20	9	9	9
Staphylococcus aureus	26	10	8	9
Salmonella	17	9	8	8

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

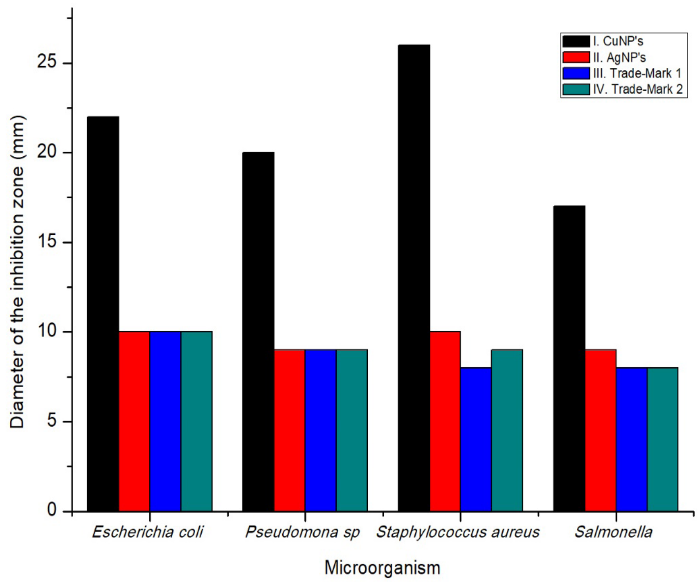

Figure 6 . Diameter of the inhibition zone in mm. After 24 h incubation.

The observed results suggest a more potent antimicrobial effect of copper nanoparticles compared to silver nanoparticles, as evidenced by the larger inhibition zones observed in all experiments. However, it is imperative to consider the potential toxicity associated with copper nanoparticles.

The precise mechanisms underlying the antibacterial action of nanoparticles remain incompletely understood. Although the exact mode of action against bacteria has yet to be fully elucidated, some researchers have proposed potential effects of nanoparticles on bacterial cells. Specifically, it is suggested that ions released by nanoparticles may bind to the bacterial cell wall, causing disruption [6]. These ions can penetrate bacterial cells, disrupting biochemical processes by generating reactive oxygen species (ROS) that interact with membrane proteins, affecting their permeability [7]. Furthermore, it is suggested that nanoparticle exposure may also impact bacterial DNA [8].

Further research is warranted to comprehensively understand the mechanisms underlying the antibacterial activity of nanoparticles and to evaluate their safety and efficacy for various applications.

5. Conclusions

The synthesis and characterization of silver and copper nanofluids have demonstrated promising antimicrobial properties against pathogenic microorganisms, including *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas* sp., and *Salmonella*. The nanofluids, particularly those containing copper nanoparticles, exhibited significant inhibition zones in antimicrobial susceptibility tests, suggesting their potential as effective disinfectants.

These findings highlight the importance of exploring alternative antimicrobial strategies amidst rising concerns over antimicrobial resistance and the limitations of traditional antibiotics. By leveraging the unique physicochemical properties of metallic nanoparticles, such as high surface area to volume ratio and biocompatibility.

However, further research is necessary to elucidate the precise mechanisms underlying the antimicrobial activity of these nanoparticles and to assess their safety and efficacy for widespread use.

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