

Research Article

Synthesis of Copper Nanoparticles by Thermal Decomposition and Their Antimicrobial Properties

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Copper nanoparticles were synthesized by thermal decomposition using copper chloride, sodium oleate, and phenyl ether as solvent agents. The formation of nanoparticles was evidenced by the X-ray diffraction and transmission electron microscopy. The peaks in the XRD pattern correspond to the standard values of the face centered cubic (fcc) structure of metallic copper and no peaks of other impurity crystalline phases were detected. TEM analysis showed spherical nanoparticles with sizes in the range of 4 to 18 nm. The antibacterial properties of copper nanoparticles were evaluated *in vitro* against strains of *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The antibacterial activity of copper nanoparticles synthesized by thermal decomposition showed significant inhibitory effect against these highly multidrug-resistant bacterial strains.

1. Introduction

Recently metallic nanoparticles (NPs) have attracted great interest because of their unique physical and chemical properties. Their properties can be controlled depending on the synthesis method. One of the main effects, which are enhanced by controlling particle size, is their antimicrobial action [1, 2]. The antimicrobial activity of NPs is known to be a function of surface area in contact with microorganisms. For this reason, high surface area NPs assure a wide range of reactions on the surface of microorganisms, inhibiting the normal function of cells or causing cell death [3].

Several methods have been used for the formation of copper nanoparticles. One of the first reported methods to obtain inorganic NPs is the chemical coprecipitation method, which involves nucleation and growth during the same process [4], but the main disadvantage of this process is the formation of agglomerated NPs with a wide size distribution. Nanometric particles with size control have been obtained through homogeneous precipitation reactions, which involve

separation of nucleation and growth process. Under conditions of homogeneous precipitation, an abrupt nucleation occurs when reactive concentrations reach a supercritical saturation point [5], generating this way a homogeneous growth by solutes diffusion from solution to surface, achieving their final size. The key to obtain homogeneous particle size is the separation of nucleation and growth steps, avoiding nucleation during NPs growth step; if performed with direct heating of the mixture, a wide size distribution of NPs can be produced [5, 6].

Thermal decomposition of metallic precursors in presence of organic surfactants at high temperature is a method widely used due to its ease to produce highly crystalline NPs with size distribution control [5, 7–11]. In the particular case of Cu NPs, low redox potential causes surface oxidation which decreases their antimicrobial characteristics, this is due to the fact that metallic copper (Cu^0) is more reactive than copper oxide [12]. Therefore, in order to obtain Cu NPs with high antimicrobial properties, the size control of NPs and the presence of a surface coating to prevent their oxidation are

very important. Lately a straightforward approach to oxide-free Cu NPs by thermal decomposition of a copper precursor has been reported for industrial applications [13].

Although only a few studies have described the antibacterial properties of copper nanoparticles, they show that copper nanoparticles have a significant potential as bactericidal agents. Hence, in this work we report the synthesis of copper NPs by thermal decomposition using phenyl ether and oleic acid, which act like an organic agent to control the growth of NPs. The obtained Cu NPs were tested *in vitro* against the bacterial strains *S. aureus* and *P. aeruginosa*, which are some of the main pathogens causing nosocomial infections worldwide [14].

2. Experimental

Copper chloride, oleic acid, and phenyl ether were provided by Sigma Aldrich; sodium oleate was provided by TCI; hexane and ethanol were provided by J. T. Baker. All reactants were used as received by the supplier. In order to evaluate the antimicrobial properties against human gram negative pathogenic bacteria, we used an inoculum containing 10^5 CFU mL⁻¹ (colony forming units per milliliter) of *S. aureus* (ATCC 6538) and *P. aeruginosa* (ATCC 13388), in Mueller Hinton broth provided by BD Bioxon.

2.1. Synthesis of Copper Nanoparticles. In a typical synthesis, 1.08 g of copper chloride and 3.65 g of sodium oleate were dissolved in a mixture of hexane, ethanol, and distilled water. The solution was heated and refluxed during 4 h, and later it was transferred to a separation funnel to eliminate the aqueous residues. The organic phase with copper-oleate complex and hexane was washed three times with distilled water. Afterwards the copper-oleate complex was transferred to a Petri dish to help evaporate the residual solvent. Next 3.6 g of copper-oleate complex was mixed with 1.14 g of oleic acid and 20 g of phenyl ether at room temperature. The solution was heated at 250°C for 30 min. During the course of the reaction, the solution turned brown in color indicating the formation of Cu NPs. The resulting mix was cooled at room temperature and the precipitate was washed a few times with ethanol to eliminate solvent and residues; finally the NPs were collected by centrifugation.

2.2. Characterization of Copper Nanoparticles. XRD patterns were obtained using a diffractometer Siemens D-500 (CuK α , 25 mA, 35 kV). Nanoparticles size was measured by means of a transmission electron microscope JEOL Titan. FTIR spectra of copper NPs were recorded in KBr pellets on Nicolet Magna-IR Spectrometer model 550.

2.3. Antimicrobial Assays. The antimicrobial activity of Cu NPs was tested against *P. aeruginosa* (ATCC 13388) and *S. aureus* (ATCC 6538). The assays were set by preparing serial dilutions containing Cu NPs at concentrations of 200, 400, 800, 1600, and 3200 μ g mL⁻¹, which were suspended in Mueller Hinton broth by sonication. Each dilution of NPs was added to 10^5 CFU mL⁻¹ of bacterial suspension

and incubated at 37°C during 16 h and 200 rpm. After that time, bacterial growth was determined by optical density measurements at 600 nm to obtain the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC). Inhibition rates were calculated by using the following equation:

$$\text{inhibition (\%)} = \frac{A_0 - A}{A_0} * 100, \quad (1)$$

where A_0 is the absorbance of the control and A is that of the test sample.

The Cu NPs antibacterial effect was determined using a medium nutrient agar adding 800, 1600, and 3200 μ g mL⁻¹ of NPs. Next a 100 μ L solution containing *P. aeruginosa* and/or *S. aureus* incubated at 37°C during 16 h was added to the growth medium containing the three concentrations. The medium was streaked with a sterile loop across the surface of the agar. The dishes were incubated at 37°C during 16 h to allow bacteria to reproduce.

3. Results and Discussion

Figure 1 shows XRD patterns of copper nanoparticles obtained by thermal decomposition of copper-oleate complex. In comparison to a copper standard (JCPDS 04-0836), the characteristic diffraction peaks of copper located at 43.7°, 50.7°, and 74.3° were observed. They correspond to the (111), (200), and (220) planes of the fcc structure, respectively. No other impurity peak was detected in the sample. This result confirms that with this process it is possible to obtain pure copper NPs.

The FTIR spectrum of copper nanoparticles obtained by thermal decomposition process is presented in Figure 2. This analysis was used to determine the functional organic groups in the surface of the nanoparticles generated by oleic acid. Two bands at 2901 and 2838 cm⁻¹ can be seen, and they are attributed to symmetric and asymmetric stretching of CH₂ group and terminal groups -CH₃ and =CH which correspond to oleic acid. Other absorption bands of carboxylate coordination and metals located at 1623 and 1450 cm⁻¹ for asymmetric stretching COO⁻ and bands located at 1123 and 847 cm⁻¹ corresponding to symmetric stretching of COO⁻ group can be seen as well [15]. Similar results using this method were reported by Roca et al. [7].

TEM micrograph of copper nanoparticles and their size distribution is shown in Figure 3(a), which displays spherical NPs with sizes below 20 nm. The distribution uniformity of NPs due to oleic acid chemically bound at their surface is also shown. The particle size distribution (Figure 3(b)) was obtained by measuring the diameter of the particles from different parts of the grid for an average number of particles close to 300. The particle size ranges between 4 and 19 nm with an average size of 9 nm.

Comparing these results with those generated by other scientists [15], the particle size obtained by this process is smaller (± 9 nm). The presence of an organic compound such as oleic acid allows particle size to be controlled and at the same time avoids its agglomeration and oxidation. This

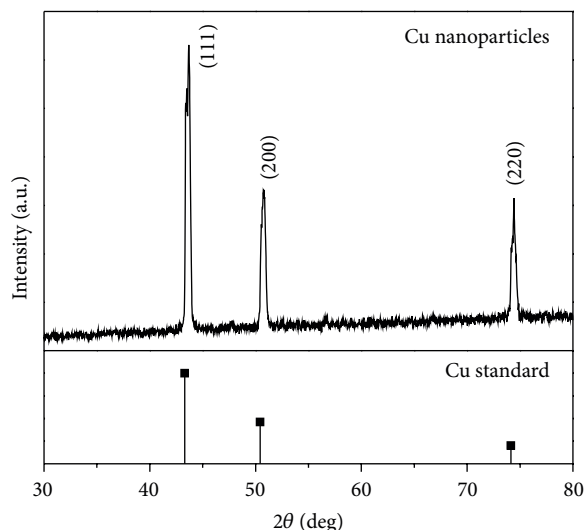


FIGURE 1: XRD patterns of Cu nanoparticles obtained by thermal decomposition and Cu standard (JCPDS 04-0836).

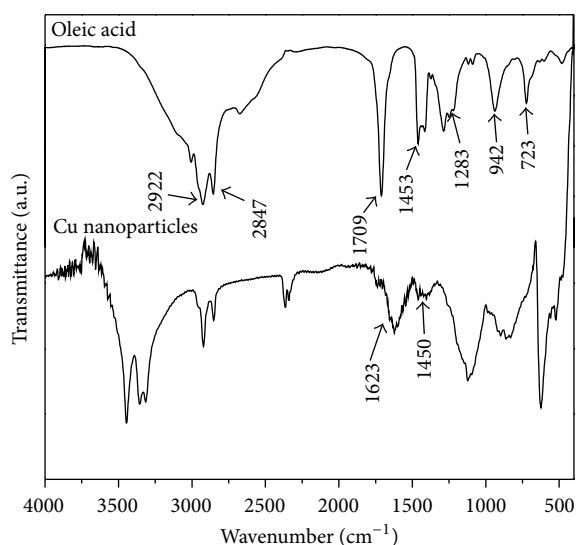


FIGURE 2: FTIR spectrum of copper nanoparticles.

synthesis method depends on the high reactivity of copper complex as well as on its concentration and agitation speed during the reaction. Both factors are very specific for controlling the morphology, particle size, and their distribution.

The antimicrobial activity of Cu NPs against the bacterial strains *S. aureus* and *P. aeruginosa* is shown in Table 1. It is evident that for both bacteria there was an increase in the inhibitory activity (%) as the Cu NPs concentration increased. With $800 \mu\text{g mL}^{-1}$ a noticeable antibacterial effect was attained on *P. aeruginosa* (99.9%), however at the same dose no inhibition at all (0%) was detected on *S. aureus*. A higher concentration ($1600 \mu\text{g mL}^{-1}$) reported similar inhibitory effect (99% and 100%) against *P. aeruginosa* and *S. aureus*, respectively. The highest dosage evaluated ($3200 \mu\text{g mL}^{-1}$) also caused a total inhibition (100%) on

TABLE 1: Copper nanoparticles concentrations tested for their *in vitro* inhibitory (%) effect on bacterial strains of *S. aureus* and *P. aeruginosa*.

Concentration ($\mu\text{g mL}^{-1}$)	Inhibition (%)	
	<i>S. aureus</i>	<i>P. aeruginosa</i>
3200	99.6	100
1600	99	100
800	0	99.9
400	0	10.3
200	0	0

P. aeruginosa, demonstrating its bactericidal effect. However, with the same dose *S. aureus* reported 99.6% inhibition. This outcome suggests that the quantity of NPs in the solution was not enough to display a full antibacterial activity against this bacterial strain. This outcome is similar to that of Cioffi et al. [16] where they documented the bacteriostatic properties of copper NPs on *S. aureus*. In a similar study Ruparelia et al. [1] tested the antimicrobial properties of silver and copper NPs against *E. coli* (four strains), *B. subtilis*, and *S. aureus* (three strains). Their studies with *E. coli* and *S. aureus* revealed greater effectiveness of the silver Nps compared to copper NPs; however, *B. subtilis* showed the highest sensitivity to NPs compared to the other strains and was more negatively affected by the copper NPs.

The *in vitro* determination of MBC of Cu NPs at each of the five tested concentrations is shown in Figures 4 and 5. Our results point out that *S. aureus* and *P. aeruginosa* were inhibited at 99.6 and 100%, respectively, with the $3200 \mu\text{g mL}^{-1}$ dose. Concentrations at 200 and $400 \mu\text{g mL}^{-1}$ did not inhibit the growth of *S. aureus* and displayed only a minor antibacterial effect (10.3%) on *P. aeruginosa*. For this reason, the bacterial sensitivity to Cu NPs was found to vary depending on the microbial species. Similar results regarding the antimicrobial effect of Cu NPs against bacterial and fungal strains have been demonstrated by Ramyadevi et al. [17]. They reported the antimicrobial activity of Cu NPs against *Micrococcus luteus*, *S. aureus*, *E. coli*, *Klebsiella pneumoniae*, and *P. aeruginosa* and on several fungi like *Aspergillus flavus*, *A. niger*, and *C. albicans*. The Cu NPs showed more inhibitory activity in bacteria than on fungi.

The observed antimicrobial effect of Cu NPs is not merely due to their release of metal ions but can also be attributed to their morphology, mainly their small size and high surface area to volume ratio, which allows them to interact closely with microbial membranes of each bacterium [18]. In a similar way the antibacterial activity of silver NPs has been reported on the same bacterial strains [19]. The *in vitro* antibacterial effect of Cu NPs observed in this work is shown in a series of pictures; Figure 4 clearly shows that *P. aeruginosa* was more sensitive to the effect of Cu NPs compared to *S. aureus*, since with $1600 \mu\text{g mL}^{-1}$ dose the antibacterial effect was evident on Petri dishes.

On the other hand, Figure 5 clearly shows that a concentration of $3200 \mu\text{g mL}^{-1}$ was required to completely inhibit the bacterial growth of *S. aureus*. This outcome points out

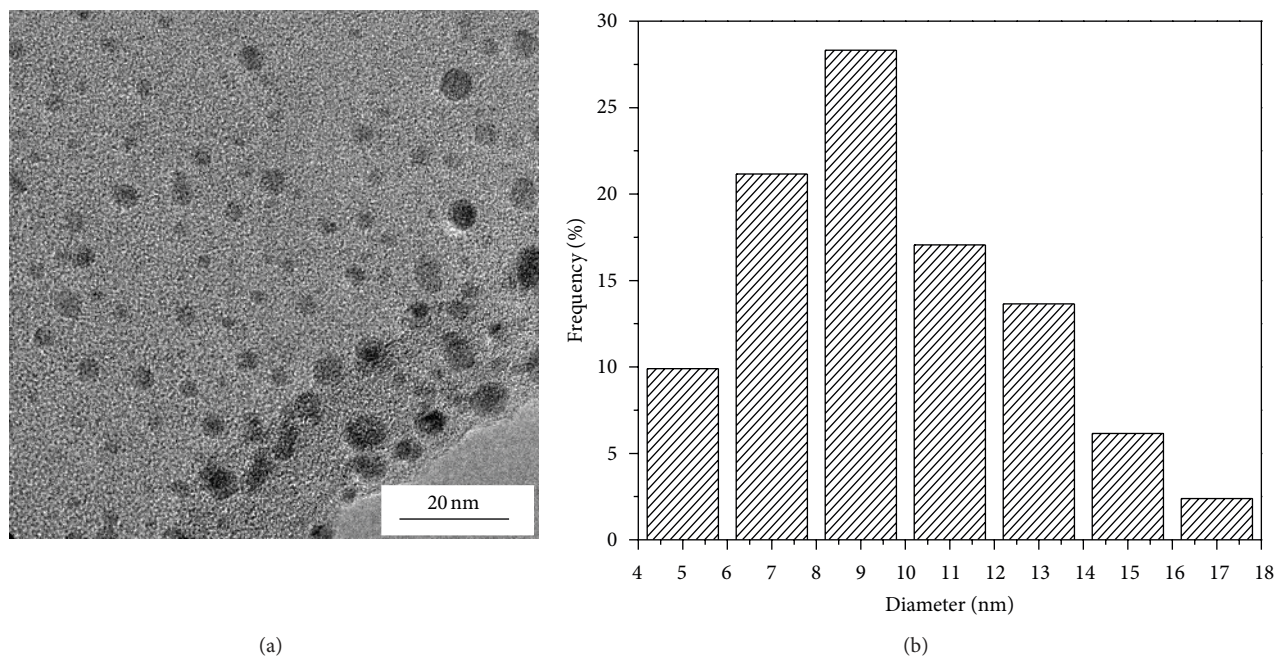


FIGURE 3: TEM micrograph of copper nanoparticles obtained by thermal decomposition (a) and size distribution histogram (b).

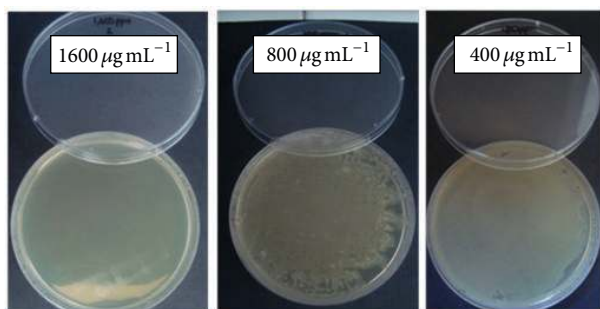


FIGURE 4: Antibacterial *in vitro* effect of copper nanoparticles at three concentrations on *P. aeruginosa*.

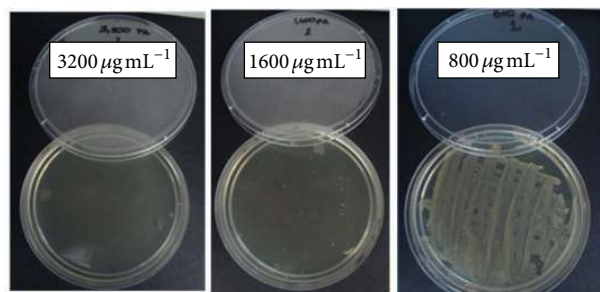


FIGURE 5: Antibacterial *in vitro* effect of copper nanoparticles at three concentrations on *Staphylococcus aureus*.

that this strain is more tolerant to the *in vitro* antibacterial effect of Cu NPs in comparison to *P. aeruginosa*, given that only 50% of copper nanoparticles concentration was required in order to inhibit any propagation of CFU. The antibacterial

effect of copper oxide nanoparticles against *S. aureus* and *E. coli* has been also demonstrated by Ren et al. [20] with similar concentrations ranging from 100 $\mu\text{g mL}^{-1}$ to 5000 $\mu\text{g mL}^{-1}$.

According to the literature, the better inhibitory effect observed in *P. aeruginosa* than in *S. aureus* is related to the difference in the outer casing of these bacteria [19, 21]. A Gram positive bacterium, as *S. aureus*, has a thick layer of peptidoglycan (a sugar-protein shell) that the Cu ions can penetrate. A Gram negative bacterium, as *P. aeruginosa*, has an outer membrane covering the thin layer of peptidoglycan on the outside. This outer membrane prevents the Cu ions from penetrating. The copper ions released by the nanoparticles may attach to the negatively charged bacterial cell wall and rupture it, thereby leading to protein denaturation and cell death [3].

4. Conclusions

Copper spherical nanoparticles in the range of 4 to 18 nm synthesized by thermal decomposition were evidenced by the X-ray diffraction and transmission electron microscopy. Copper nanoparticles showed an inhibitory effect against *S. aureus* (99.6%) and *P. aeruginosa* (100%) with 3200 $\mu\text{g mL}^{-1}$, respectively. Therefore according to the results, we can conclude that copper metallic nanoparticles have a commercial potential to be used in public health issues, for prevention of food spoilage and for agricultural purposes.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

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