Original Article

Synthesis of ferrite nanoparticles Cu$_{1-x}$Ag$_x$Fe$_2$O$_4$ and evaluation of potential antibacterial activity

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A B S T R A C T
Copper-silver ferrite nanoparticles synthesized by a simple and rapid solution combustion method using glycine as complexing agent. Cu-Ag nanoparticles were and characterized by X-ray diffraction and scanning electron microscopy. In vitro assays were established as a means of evaluating the potential of interaction and mean inhibition rates on several strains using the diffusion method in liquid broth medium. Ultraviolet-visible spectroscopy analyses were applied for comparing relative amounts of strains eliminated or inhibited through physical sample mechanisms. Results indicated that the amount of silver ions in the copper ferrites influences the crystal microstructure and antibacterial properties of the copper ferrites.

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1. Introduction

Microorganism resistance to antibiotic treatments has increased in recent decades, becoming a concern due to potential harmful effects on human health. As a result, increasing occurrences of genes with higher resistance to antibiotics have been detected in certain bacteria that come in contact with humans and other animals species [1]. According to Raffi et al. [2], such a situation is even more serious when the immunity of certain microorganisms stems from the indiscriminate use of antibiotics against any pathogen, resulting in side effects that reflect growing bacterial resistance to many antibiotics.

Considering the need to find new alternatives for the control of bacterial and fungal proliferations in uncontrolled environments, several studies have been published using concepts regarding the interaction of nanostructured materials and microorganisms, studying the possible effects of this contact [3–6].

The use of silver metal particles as antibacterial agents is noteworthy, due to their advantages in terms of chemical stability, resistance to temperature variations, efficacy and

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long-term durability. These advantages can be extended by considering the relatively low toxicity of these particles to the human body compared to other inorganic metals [5]. An example of the attractiveness of these nanoparticles is applications in water-purifying systems, widely diffused in the industry.

In this context, the aim of the present study is to evaluate the performance of copper-silver ferrite nanoparticles as bactericidal material, as well as evaluate the interaction and inhibition rates caused by exposure to microbes. Functional nanoparticles with superparamagnetic and antibacterial properties were obtained through the solution combustion method, aiming viable options in the control against Staphylococcus aureus (S. aureus), Pseudomonas aeruginosa (P. aeruginosa), Bacillus subtilis (B. subtilis), Escherichia coli (E. coli), Klebsiella pneumoniae (K. pneumoniae) and Enterococcus faecalis (E. faecalis).

2. Materials and methods

2.1. Solution combustion synthesis

Cu-Ag ferrite nanoparticles were synthesized by a modification of the simplified solution combustion method reported by Toniolo et al. [7]. Briefly, a stoichiometric mixture of cupric nitrate, silver nitrate and ferric nitrate was diluted with the reducing agent glycine (C2H5NO2) in a vessel containing deionized water, at masses previously defined for the desired composition of the final product and in the stoichiometry for the complete consumption of the oxides by the complexing agent [8]. Two ratios between the reducing agent and the oxidizing agents were evaluated, fixing the glycine nitrate (G:N ratio) molar ratio at 1:1 and 2:3.

The reaction components were then heated to 100 °C until an exothermic redox reaction between the fuel and the oxidizing agent occurred homogeneously. This solution was brought almost instantaneously to the self-sustained simultaneous combustion process in the entire solution volume, resulting in a high volume powder with dark precipitates, defined by the amount of oxide present in the samples.

2.2. X-ray diffraction (XRD) characterization

The ferrite samples were characterized by a powder X-ray diffractometer (Panalytical X’Pert Pro), using Cu-Kα radiation (λ = 1.5405 Å) at 40 kV and 35 mA. Rietveld refinements of the experimental patterns were performed using the software Highscore Plus 3.0.

The average size of the crystallites in a sample can be estimated from X-ray diffraction measurements using the Williamson-Hall plot based in the classic Debye-Scherrer method [9], considering the full width at half maximum of the characteristic diffraction peaks to determine the crystallite size, according to Eq. (1)

$$ \beta \cos \theta = \frac{0.9 \lambda}{L} + c_r \sin \theta $$

where λ is the wavelength of the Cu-Kα incident radiation and c_r sinθ corresponds to a lattice strain.

### Table 1 - Copper-silver ferrite samples evaluated in the present study.

<table>
<thead>
<tr>
<th>Structural sample composition</th>
<th>G:N molar ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>C510 – Cu0.95Ag0.05Fe2O4</td>
<td>1:1</td>
</tr>
<tr>
<td>C507 – Cu0.95Ag0.05Fe2O4</td>
<td>2:3</td>
</tr>
<tr>
<td>C210 – Cu0.98Ag0.02Fe2O4</td>
<td>1:1</td>
</tr>
<tr>
<td>C207 – Cu0.98Ag0.02Fe2O4</td>
<td>2:3</td>
</tr>
</tbody>
</table>

In this way the lattice parameters can be calculated for all samples by means of Eq. (2), as follows,

$$ a = d_{hk1} \sqrt{h^2 + k^2 + l^2} $$

where d_{hk1} is the interplanar spacing of the peak relative to the crystallographic plane under analysis.

2.3. Preparation of the culture medium and strain selection

Gram-positive strains S. aureus, B. subtilis and E. faecalis and Gram-negative P. aeruginosa, E. coli and K. pneumoniae were selected for quantitative bioassay analyses with ferrite samples Cu0.95Ag0.05Fe2O4 and Cu0.98Ag0.02Fe2O4.

With the aid of an inoculating loop, a fragment of the preserved bacterium was removed and transferred to a Petri dish containing Mueller–Hilton Agar culture medium. After incubating for 24 h, an aliquot of the bacteria was transferred into a test tube containing 5.0 mL of Mueller–Hinton broth culture medium. All tubes were then incubated for 4 h at 37 °C for bacteria activation and synchronization.

A total of 1.0 mL of the grown bacteria was transferred to a tube containing 1 mL of 10% formaldehyde. The number of colonies was measured at 625 nm on a UV–vis spectrophotometer (Thermo Spectronic Genesys 10 UV). The concentrations were then adjusted between 10^6 – 10^7 CFU/mL in tubes containing 5.0 mL of Mueller–Hinton broth medium.

2.4. Sample separation and incubation

Cu-Ag ferrites with structural composition Cu0.95Ag0.05Fe2O4 and Cu0.98Ag0.02Fe2O4, at silver ion concentrations of 2% and 5%, respectively, were tested. The copper-silver samples underwent molecular modifications during the synthesis step, adjusting a higher amount of oxidants per reagent (G:N ratio of 1:1 and 2:3), in order to investigate possible effects induced by changes in the stoichiometry of the reaction in the process of bacterial activity mitigation.

The ferrite samples were separated according to their chemical structure, in order to investigate bacterial growth inhibitory processes and the Ag⁺ ion ratios in these processes. To this end, four Cu-Ag ferrite samples were used, described in Table 1.

Sample masses were selected for the concentrations of 250 μg/mL and 500 μg/mL. A volumetric concentration of 5.0 μL of bacterial culture at 10⁷ CFU/mL were added to the samples, adjusted by the growth density in each culture.

Each sample was homogenized a total of three times with the culture medium to remove large aggregates. The samples were then maintained under incubation conditions (Edmund
Bühler TH-30) with axial shaking at 115 rpm at 36–37 °C, overnight. All assays were performed in triplicates to ensure total reproducibility and reliability.

3. Results and discussion

3.1. Microstructural properties

The XRD patterns confirm data reported in the literature [5,10] for copper ferrites in the presence of a silver oxide phase. The crystallographic indices (2 2 0), (3 1 1), (2 2 2), (4 0 0), (4 2 2), (5 1 1), (4 4 0) and (5 3 3) with diffraction angles between 30° and 75° are displayed in Fig. 1, corresponding to the characteristic peaks of CuFeO₄, indicating a cubic spinel crystalline structure. Several diffraction peaks indicating the presence of the silver oxide (Ag₂O) are also shown, corroborating previous results reported in the literature.

Crystallographic data of copper ferrites and silver oxide were used to determine the characteristic peaks, and the adjustment was solved using Eq. (1) by the comparison between Rietveld method and analytical methods. The interplanar spacing \(d_{hkl}\) determined by means of the mean position of the diffraction peaks and net parameters were then deduced from Eq. (2). The mean crystallite size was obtained from the higher intensity peaks of the Bragg reflections given by Rietveld adjustment, showing about 13 nm for the 98% copper nanoparticles and 11 nm for the 95% copper sample.

Table 2 displays the crystallographic analysis of the parameters provided by the diffraction pattern of the Cu₀.₉₈Ag₀.₀₂Fe₂O₄ and Cu₀.₉₅Ag₀.₀₅Fe₂O₄ structures. The experimental peak intensities were compared to values reported in the literature [5,10,12].

One of the possibilities of the reduction observed in the lattice parameters with the increase of the amount of silver is due to the compression of the spinel network by the metallic silver phase formed in the limits of the particle [5,11]. This factor is also reflected in the average crystallite size, which tends to be statistically lower for larger Ag⁺ ion distributions in the range of \(0 < x_{Ag} < 0.1\).

Scanning electron microscopy (SEM) micrographs of the Cu-Ag nanoparticles are displayed in Fig. 2. The average size of the Cu₀.₉₅Ag₀.₀₅Fe₂O₄ nanoparticles was of approximately 75 ± 13 nm, while for the Cu₀.₉₈Ag₀.₀₂Fe₂O₄ samples size was of approximately 69 ± 17 nm.

According to results published by Gong et al. [6] in silver-doped magnetite (Fe₂O₄@Ag) samples, Ag⁺ ions tend to initially cluster on the nanoparticle surfaces, resulting in larger and more spherical particles.

### Table 2 – Crystallographic parameters of the Cu-Ag nanoparticles analyzed in Fig. 1.

<table>
<thead>
<tr>
<th>Cu₀.₉₈Ag₀.₀₂Fe₂O₄</th>
<th>Cu₀.₉₅Ag₀.₀₅Fe₂O₄</th>
</tr>
</thead>
<tbody>
<tr>
<td>(2\theta)</td>
<td>h kl</td>
</tr>
<tr>
<td>30.63</td>
<td>2 2 0</td>
</tr>
<tr>
<td>36.42</td>
<td>3 1 1</td>
</tr>
<tr>
<td>43.57</td>
<td>4 0 0</td>
</tr>
<tr>
<td>54.03</td>
<td>4 2 2</td>
</tr>
<tr>
<td>57.77</td>
<td>5 1 1</td>
</tr>
<tr>
<td>63.28</td>
<td>4 4 0</td>
</tr>
<tr>
<td>74.72</td>
<td>5 3 3</td>
</tr>
</tbody>
</table>

Fig. 1 – Ferrite diffraction pattern (a) Cu₀.₉₈Ag₀.₀₂Fe₂O₄ and (b) Cu₀.₉₅Ag₀.₀₅Fe₂O₄.
Regarding copper ferrite nanoparticle morphology analysis by SEM, the average diameter of the particles is of approximately 68 ± 18 nm, corroborating the fact that the particles become larger and more uniform with higher amounts of silver added to the Cu-Ag samples.

3.2. Data collection and evaluation of antibacterial properties

The spectrophotometric data was relevant to associate the inhibitory potential of the particles against the previously mentioned bacteria classes. Optical density measurements allowed for counting of the previous and remaining colonies, determining viable cell rates after contact with the bacteria.

Results indicate that Cu$_{0.95}$Ag$_{0.05}$Fe$_2$O$_4$ (C510 and C507) ferrite samples display high bioactivity against most of the bacteria tested herein, and completely inhibit colony growth at higher concentrations.

On the other hand, Cu$_{0.98}$Ag$_{0.02}$Fe$_2$O$_4$ (C210 and C207) samples do not present high inhibition potential at lower concentrations (Fig. 3). However, by increasing sample concentrations in the medium to 500 µg/mL, they act significantly, inhibiting the growth of Gram positive bacteria by around 93% and Gram negative bacteria by approximately 82%.
Copper ferrites without the addition of the silver phase were also submitted to the test, but were not sufficient for inhibitory action against the evaluated microorganisms, thus allowing for their proliferation. This factor is strongly indicative that the affinity of the silver ions, alongside the strong magnetostatic properties of the copper nanoparticles, displays a clear influence on the antibiotic parameters of the samples.

Changes in the stoichiometry of ferrite Cu$_{0.99}$Ag$_{0.01}$Fe$_2$O$_4$ during its synthesis indicate the tendency for increased bactericidal properties (Fig. 4). This effect can be understood by increasing the contact surface area ratio between the samples and the microorganisms, subjecting them to a larger amount of agent per volume of solution. Several studies have indicated that metallic nanoparticles display antibacterial behavior characteristics depending on the distribution and size of the agglomerates [4,5,11–13].

According to Raffi et al. [2] and Sanpo et al. [12], there are a number of possible mechanisms for the antibacterial action of copper nanoparticles, such as those treated herein. Studies suggest that when E. coli is treated with copper nanoparticles, the major changes occur in the morphology of its cell membrane. These particles adhere to the surface of the bacterial wall by strong adsorption and penetrate through the cell membrane due to the action of the copper ions, which promotes degradation and disappearance of cytoplasm, destruction of the bacterium cell wall, leading the cell to death [2,14,15]. Thus, it is also proposed that silver ions associated with copper nanoparticles possibly lead to microorganism cytotoxicity, which increases their bioactive influence and makes them effective bactericidal compounds [2,4,6].

As nanoparticles have a large surface area, the biocidal efficacy of the Cu-Ag ferrites, thus, lies in the increased surface-to-volume ratio of the samples compared to other types of morphologically larger particles [12,16].

4. Conclusions

Copper-silver ferrite nanoparticles have been prepared and synthesized by a simplified homogeneous solution combustion process using glycine as complexing agent. This method can produce fine and functional iron oxide particles easy and quickly when compared to other synthesis routes. The samples were characterized using X-ray diffraction. Rietveld refinement analysis shows a spinel structure for copper-silver ferrites with mean crystallite sizes ranging from 11 to 13 nm. A compressive tension can be observed with the increase of silver, which strongly changes the biocidal activity of the samples. In addition, scanning electron microscopy shows that the nanocrystalline Cu$_{1-x}$Ag$_x$Fe$_2$O$_4$ produced exhibits a spherical shape and has high surface-to-volume ratio relative to amount of silver ions in the structure.

The efficacy of the antibacterial action was tested against both Gram positive and Gram negative bacteria strains, and an increasing inhibitory tendency with the addition of silver ions in the copper ferrite sites was observed, suggesting that silver ions are fundamental in enhancing the biocidal action of ferrites.

The data also indicate that the action of silver as a carrier mechanism becomes essential in enhancing the bioactivity of the nanoparticles against the tested microorganisms.

Further studies are underway with the aim of verifying the feasibility of particle reuse after treatment by magnetic removal. Analyses regarding the antimicrobial activity will be extended against other resistant microorganisms.

Conflicts of interest

The authors declare no conflicts of interest.

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References


