

Synthesis of copper nanostructures on silica-based particles for antimicrobial organic coatings



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ABSTRACT

Sol-gel based silica nanoparticles of 100 nm were used to interact with copper ions from the dissolution of CuCl_2 allowing the synthesis of paratacamite ($\text{Cu}_2(\text{OH})_3\text{Cl}$) nanocrystals of around 20 nm. The method produced well dispersed copper nanostructures directly supported on the surface of the SiO_2 particles and was generalized by using a natural zeolite microparticle as support with similar results. These hybrid Cu based nanoparticles released copper ions when immersed in water explaining their antimicrobial behavior against *Escherichia coli* and *Staphylococcus aureus* as measured by the minimum inhibitory and minimum bactericidal concentrations (MIC and MBC). Noteworthy, when these nanostructured particles were mixed with an organic coating the resulting film eliminated until a 99% of both bacteria at concentrations as low as 0.01 wt%.

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1. Introduction

Synthesis of metal based nanoparticles on the surface of a supporting particle is a relevant approach producing nanomaterials with improved characteristics such as stability and reactivity [1,2]. Metal nanoparticles supported on metal oxides and carbon surfaces are the basis for many catalysts of importance in energy technologies, pollution prevention, and environmental cleanup [2]. From the different kinds of supported metal nanoparticles, those having copper are highlighted due to their wide range of applications, especially in catalysts [3–10]. Dispersed copper catalysts have been of great interest in several reactions from steam reforming to biosensing applications [11]. However, copper nanoparticles also emerge as an active and strong antimicrobial agent that can be supported on different nanostructures toward the development of novel biocide materials [12–16]. Noteworthy, copper nanoparticles can be embedded in polymer matrices allowing the development of novel antimicrobial polymeric materials [17–20]. The relevance of nanotechnology in this area relates with larger release of the active agent (Cu^{2+}) from nanoparticles than from microparticles [21,22]. Moreover, by supporting copper nanoparticles the aggregation is decreased avoiding the deterioration of its chemical properties and therefore of its antimicrobial behavior [14]. For instance, a direct relationship between nanoparticle dispersion and release of metal

ions was found in polymer/copper composites [23]. Moreover, the release of the active agent is delayed when these particles are supported [14]. Therefore, the ion release from copper nanoparticles can be controlled by a proper support design.

From the different kinds of copper particles, stoichiometric $\text{Cu}_2(\text{OH})_3\text{Cl}$ basic copper(II) chloride is stressed due to its unique chemistry and physical properties. $\text{Cu}_2(\text{OH})_3\text{Cl}$ is known to have four types of crystal structures, including orthorhombic atacamite, monoclinic botallackite, rhombohedral paratacamite, and monoclinic clinoatacamite [24]. For instance, $\text{Cu}_2(\text{OH})_3\text{Cl}$ paratacamite can be used in a wide range of applications such as a catalysts [25], corrosion prevention [26], agriculture [27], and hydrogen uptake and storage [28]. $\text{Cu}_2(\text{OH})_3\text{Cl}$ was superior to the traditional $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ for reoxidizing reduced palladium catalysts [26]. Unlike botallackite [29], nanoparticles based on paratacamite have not been published focusing on their support neither on its antimicrobial activity [24,25,28,30–32]. These nanostructures are synthesized from copper salt solutions through pH regulation and, for instance, paratacamite is usually produced from copper chloride solution under basic condition [24]. Other methods based on hydrothermal and heat-treatment reactions have also been reported using urea as reagent [28].

Based on the above mentioned, the goal of the present manuscript was to synthesize paratacamite nanostructures on the surface of silica-based particles. These supported nanostructures were able to both release copper ions when immersed in water and eliminate bacteria. Moreover, these nanoparticles were used to produce antimicrobial organic coatings.

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2. Experimental

2.1. Synthesis of nanoparticles

Spherical silica nanoparticles of 100 nm were synthesized by the sol–gel method using a two-stage mixed semi-batch method, as previously reported [33,34]. In the first stage, Solutions A and B were prepared. For Solution A, 0.5 M of tetraethylortosilicate (TEOS) was dissolved in 22 mL of ethanol whereas in Solution B, 0.2 M of aqueous ammonia solution was added to 23 mL of ethanol and 2 mL of water. Solution B was then added dropwise to Solution A. The resulting mixture was allowed to react for 60 min at 40 °C under a N₂ atmosphere. In the second stage, Solutions A and B were again prepared and added to the reactor containing the solution and the particles prepared in the first stage. The solutions were separated by centrifugation (10,000 RPM, 20 min) and washed three times with ethanol. The methodology used for the synthesis of paratacamite nanostructures was based on the results from Kim et al. [14]. In particular, 1.56 g of CuCl₂ (from Sigma–Aldrich), 0.5 mL of ammonia solution (from Sigma–Aldrich) and 1.5 g of silica nanoparticles were mixed in 100 mL of deionized water during 6 h at pH 4. Afterward, the solution was filtered and dried at room temperature. The same procedure was used for zeolite microparticles. In this case, a natural mordenite was used.

2.2. Particle characterization

The morphology of the particles was characterized by a FEI microscope model G2 F20 S-Twin high-resolution transmission electron microscopy (HRTEM) at 200 kV. The samples were dispersed in ethanol previous to the observation. Wide angle X-ray diffraction analysis was carried out using a Siemens D-5000 diffractometer with CuK α =1.54 Å and a step scan of 0.02° at room temperature. Elemental analyses for the particles were carried out with inductively coupled plasma mass spectrometry (ICP-MS) Neptune Plus model. The thermal stability was evaluated by calcination of obtained particles at temperature 450 °C at a heating rate of 10 °C/min during 4 h using a Thermo Fisher Scientific Thermolyne.

The release of cupric ion was measured using a UV–Visible (Model JENWAY 6320D) spectrophotometer. The ion concentration in 3 mL of sample solutions was determined by adding 1 mL of 10% hydroxylamine hydrochloride solution, 1 mL of sodium acetate–acetic acid buffer (pH 4.5) and 1 mL of 1.92 × 10⁻³ mol neocuproine (copper reagent). The neocuproine was used to form a yellow chelate with the cupric ions. Ions released from hybrid particles were determined by adding 0.05 g in 60 mL of deionized water. Before each measurement at time, the solution was centrifuged for 15 min at 19,000 rev min⁻¹.

The antibacterial activity was determined by turbidimetric method. The *Escherichia coli* (K-12 DH5 α) and *Staphylococcus aureus* (isolated strain) were tested. A pure culture of the specified bacteria was grown overnight then diluted in growth-supporting Muller Hinton broth to a concentration between 1 × 10⁶ and 1 × 10⁹ CFU/mL. A stock of tubes with a dilution of the antimicrobial substance were prepared in low-phosphate Tris-buffered mineral salts (LPTMS) medium. The dilutions were from 0 to 2000 mg/L of the particles, increasing by 125 mg/L. An aliquot of 100 μ L of bacteria was incubated in each tube overnight in a shaking incubator at 37 °C and 200 rpm. Inoculated broths without antimicrobial substance were used as blank. The tubes were then characterized by turbidity. Turbidity indicates growth of the microorganism and the MIC (minimum inhibitory concentration) is the lowest concentration where no growth is visually observed. The MIC is therefore defined as the lowest concentration of the antimicrobial substance which will inhibit the *in vitro* growth of the bacteria. An aliquot of 100 μ L of the dilution representing the MIC was incubated in a plate

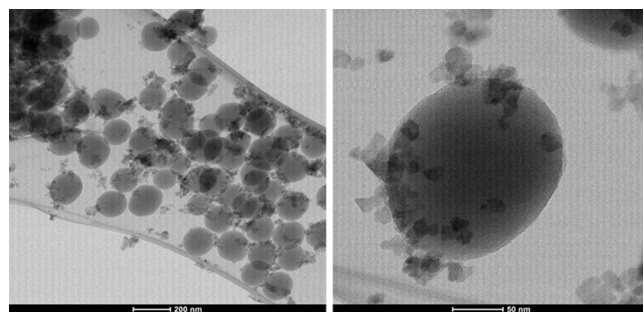


Fig. 1. TEM images for hybrid particles synthesized by using silica nanoparticles of 100 nm. Copper nanostructures are mainly formed on the surface of the silica-based particles.

and enumerated to determine viable CFU/mL. The MBC (minimum bactericidal concentration) otherwise was defined as the lowest concentration of the antimicrobial substance to kill 99.9 percent of the initial bacterial inoculum. MBC was determined from the tubes showing no turbidity. An aliquot of 100 μ L of the dilution representing the MBC was incubated in a plate and enumerated to determine viable CFU/mL. All the experiments were repeated at least twice.

2.3. Organic coating

A latex paint was used as a standard organic coating where particles were dispersed at concentrations of 0.01 and 0.1 wt%. The solution was stirred by a magnetic mixer for 10 min and the final composites were stable without any evidence of particle precipitation. After mixing, the dispersion was coated on a wood surface where a homogeneous film was obtained.

The antibacterial activity was determined by plate method. *E. coli* (K-12 DH5 α) and *S. aureus* (isolated strain) were tested. A pure culture of specified bacteria was grown overnight then diluted in growth-supporting Muller Hinton broth to a concentration between 1 × 10⁶ and 1 × 10⁹ CFU/mL. The initial concentration of bacteria was determined by incubated in a plate for 24 h at 37 °C. An aliquot of 100 μ L of the bacteria broth was poured into the wood surface and covered with a glass during 6 h at 37 °C. After the prescribed time had elapsed, the samples were thoroughly washed with 5 mL solution of 0.88 wt% NaCl and 1 wt% of Tween 80 to remove the bacteria. An aliquot of 100 μ L of the each sample was diluted 0.1, 0.01 and 0.001 in low-phosphate Tris-buffered mineral salts (LPTMS) medium. From these dilutions, a 100 μ L was incubated in a plate for 24 h at 37 °C and the bacteria colonies were enumerated to determine viable CFU/mL. All the experiments were repeated at least twice.

3. Results

Fig. 1 displays TEM images of the nanostructures synthesized using SiO₂ nanoparticles as support. Sol–gel method was able to produce well defined and homogeneous spherical silica nanoparticles with diameters around 100 ± 8 nm. Noteworthy, by adding CuCl₂ salt to a solution having these silica nanoparticles, well dispersed copper nanoparticles were obtained, as observed in **Fig. 1**. These nanostructures were mostly absorbed on the support although coexisting with unanchored particles. The method produced a mono-dispersed particle size distribution with values around 20 nm. The interaction between the metal ions and the oxide surfaces of the silica, for instance the terminal OH groups formed by dissociative adsorption of water molecules, can explain the presence of copper nanoparticles [14]. In a similar system, the formation of copper nanoparticles on a silica surface was summarized in three steps [14]: (1) deprotonation of hydroxyl ligand

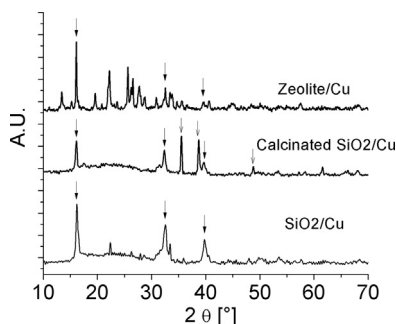


Fig. 2. X-ray diffraction patterns of the hybrid particles synthesized by using either silica nanoparticles (SiO_2/Cu) or natural zeolite particles (zeolite/ Cu). It is further shown the X-ray diffraction patterns of the SiO_2/Cu particles calcinated. Close- and open-arrows show the main diffractions peaks associated with paratacamite and tenorite structures, respectively.

from SiOH by adding base catalyst generating the $\text{SiO}-$ groups; (2) electrophilic attack by Cu^{2+} that can be easily bonded with the nucleophilic part ($\text{SiO}-$); and (3) growth of the Cu -based nanoparticles by attaching more metal ions on the surface of the SiO_2 nanoparticle. The electrostatic interactions between copper metal cations and the particle surface should be further considered as contributing to step two, as reported elsewhere [35–37].

Fig. 2 shows the X-ray diffraction patterns of the hybrid particles synthesized. Pure silica is an amorphous material displaying a broad not-defined diffraction peak around $2\theta = 20^\circ$. However, the hybrid structures display well defined peaks at $2\theta = 16.2^\circ$, 32.6° , and 39.8° , corresponding to (101), (113) and (024) planes of paratacamite ($\text{Cu}_2(\text{OH})_3\text{Cl}$) crystals [24,28]. The crystal structure of paratacamite obtained was rhombohedral. Therefore, based on TEM images and X-ray diffraction patterns, we concluded that the presence of silica nanoparticles drives the formation of copper nanostructures based on paratacamite. The thermal stability was further evaluated by heating the sample during 4 h to 450°C (calcination process) with a scan rate of $10^\circ\text{C}/\text{min}$. After calcination, a change in the X-ray pattern of the sample was observed as displayed in **Fig. 2**. Diffraction peaks at 35.6° , 38.6° , and 48.8° appeared representing to (111), (111) and (20–2) planes of tenorite crystal structure of copper oxide (CuO) [3]. At this mild calcination condition, the paratacamite structures remained. However, by TEM images were not observed copper nanostructures as those displayed in the original samples likely because of this temperature favored crystal melt and its sinterization.

To further extent this methodology, a natural zeolite microparticle (mordenite) was used as support using the same methodology as in silica nanoparticles. Zeolite materials are aluminosilicate minerals with a surface similar to those observed in pure silica: oxide metals with OH terminal groups. TEM images of these copper/zeolite hybrid materials are displayed in **Fig. 3**. The zeolite showed nanostructures on its surface with sizes even smaller than in the case of silica although with larger agglomeration due to the low specific surface area of microparticles. These particles also displayed X-ray diffraction peaks associated with paratacamite structures, as displayed in **Fig. 2**, confirming our approach toward synthesis of copper nanostructure by using silica based support.

As explained in Section 1, copper is an outstanding antimicrobial agent. The mechanisms of copper toxicity toward microorganisms are associated to several processes such as presence of radicals, displacement of essential metals from their native binding sites, or ligand interactions [38]. From the material point of view, independent of the specific mechanism, the copper ion (Cu^{2+}) is the active agent. Therefore, any material able to release copper ions above some threshold will display some antimicrobial activity [39]. In particular, higher antimicrobial behavior will be found in materials

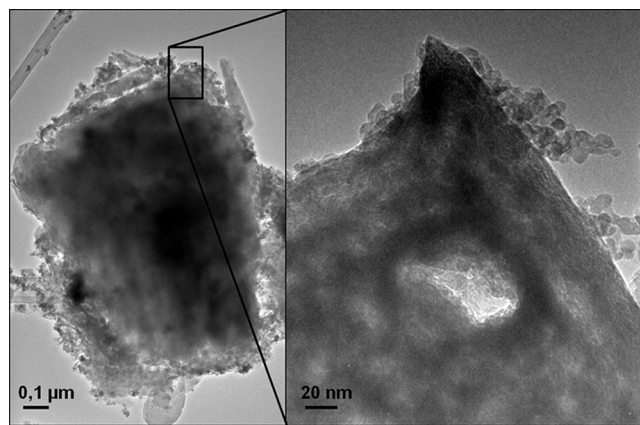


Fig. 3. TEM images for hybrid particles synthesized by using a natural zeolite microparticles. Copper nanostructures are mainly formed on the surface of the particle.

with higher ion release [19,20,40,41]. Based on this hypothesis, the copper ion release rate of these hybrid particles was tested as displayed in **Fig. 4**. This figure shows that nanostructured paratacamites supported on silica-based particles were able to release copper ions after water immersion. Moreover, this release was not immediately as expected for salt compounds but rather time-dependent as displayed in **Fig. 4**. It was observed that ions increase readily with immersion time due to hydrophilic structure of the supports. Regarding the different amount of ions released from the particles, this tendency can be explained noting the larger amount of copper presented in the hybrid zeolite particle having 20 wt% of copper ions as compared with the hybrid silica particle having 13 wt%, as determined by elemental analysis ICP. By taking into account the total amount of copper ions presented in each particle in the release experiment, we conclude that after 6 h of water immersion the 20.1 and 17.4 wt% of the original copper content was released, for hybrid zeolite and silica, respectively.

In absence of copper, the silica and zeolite are inert materials against the microorganisms. However, the hybrid particles exhibited antibacterial performance; **Table 1** shows the antimicrobial behavior of the particles against *E. coli* and *S. aureus* as measured by MIC and MBC. Independent of the support used, paratacamite nanocrystals supported on silica based particles were able to both inhibit and kill bacteria at concentrations around $1500 \mu\text{g}/\text{mL}$. This biocide behavior is related with the metal ion release as above discussed. Regarding the influence of the microorganism, several studies report that as the antimicrobial agent should cross the double membrane and the periplasmic space of Gram negative bacteria, it is more efficient the elimination of Gram positive bacteria [42,43].

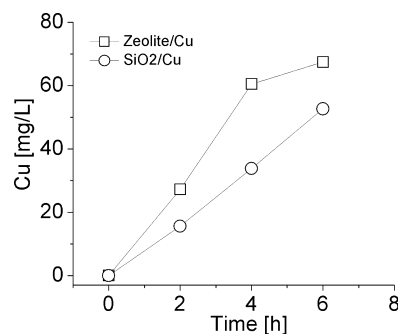


Fig. 4. Effect of time on the copper ions released from the hybrid nanostructures synthesized by using either silica nanoparticles (SiO_2/Cu) or natural zeolite particles (zeolite/ Cu).

Table 1

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of paratacamite nanostructures supported on either silica (SiO₂/Cu) or zeolite (zeolite/Cu) particles against Gram negative *Escherichia coli* and Gram positive *Staphylococcus aureus*.

Bacteria	Initial concentration [CFU/mL]	SiO ₂ /Cu			Zeolite/Cu		
		Control SiO ₂	MIC [μg/mL]	MBC [μg/mL]	Control zeolite	MIC [μg/mL]	MBC [μg/mL]
<i>E. coli</i> (N)	5.5 × 10 ⁹	>5000	1500	1650	>5000	1500	1500
<i>S. aureus</i> (P)	4.1 × 10 ⁸	>2000	1200	1350	>2000	1200	1500

N: Gram negative bacteria; P: Gram positive bacteria.

Table 2

Antimicrobial behavior of an organic coating filled with paratacamite nanostructures supported on either silica (SiO₂/Cu) or zeolite (zeolite/Cu) particles against Gram negative *Escherichia coli* and Gram positive *Staphylococcus aureus*. The bacteria reduction is defined as the ratio between the bacteria survival on the filled organic coating and the bacteria survival on the pure organic coating after 6 h of contact.

Filler and concentration	Bacteria	Bacteria reduction [%]
SiO ₂ /Cu at 0.01 wt%	<i>E. coli</i>	99.6
	<i>S. aureus</i>	99.9
SiO ₂ /Cu at 0.1 wt%	<i>E. coli</i>	99.3
	<i>S. aureus</i>	99.9
Zeolite/Cu at 0.01 wt%	<i>E. coli</i>	99.0
	<i>S. aureus</i>	99.9
Zeolite/Cu at 0.1 wt%	<i>E. coli</i>	99.0
	<i>S. aureus</i>	90.1

This is confirmed in our case as higher MIC and MBC values were obtained against Gram negative *E. coli* than Gram positive *S. aureus*.

Based on these results, it was hypothesized that paratacamite hybrid materials can be used as additive in different matrices or coatings for antimicrobial applications. To confirm this hypothesis, these nanostructured particles were mixed with an organic coating (latex paint) in order to obtain a film with filler concentrations of 0.01 and 0.1 wt%. Table 2 shows the antimicrobial results from these films against *E. coli* and *S. aureus*. Despite the low concentration of particles, these films were able to eliminate both kinds of bacteria after only 6 h of contact independent of the kind of bacteria used. Results from Table 2 showed that by increasing the amount of filler in the organic coating, the bacteria reduction is not improved. Two reasons can explain this tendency: (1) copper saturation in the aliquot added on the sample surface during the antimicrobial tests since if this saturation occurred in the sample with low amount of copper, the ion released, and therefore the antimicrobial activity, cannot be increased in samples with higher particle content; and (2) agglomeration of particles at higher concentrations decreasing the release of copper [23].

4. Conclusions

Silica based particles can be successfully used as a medium for the synthesis of copper nanostructures based on paratacamite. In particular, sol–gel based silica nanoparticles produced copper particles of around 20 nm supported on their surface whereas zeolite materials under the same conditions produced even smaller structures. These hybrid materials were able both to release copper ions after water immersion and to eliminate *E. coli* and *S. aureus* bacteria, as measured by MIC and MBC. This antimicrobial behavior could be rendered to an organic coating allowing the design of biocide films with filler concentrations as low as 0.01 wt%.

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