

Antimicrobial polymer composites with copper micro- and nanoparticles: Effect of particle size and polymer matrix

Journal of Bioactive and
Compatible Polymers

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DOI: 10.1177/0883911515578870

jbc.sagepub.com



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Abstract

The addition of metal particles, for instance, silver or copper, into polymer matrices is a relevant strategy producing novel antimicrobial materials. By using two particles with diameters around 10 nm and 45 μ m, and polymers with different characteristics, the effect of filler size and matrix on the biocide behavior of polymer/copper composites was studied. The composites were prepared by melt mixing, and the ion release from these materials was used to obtain critical information about the processes involved. Regarding the effect of the particle size, our results for polypropylene showed that this variable drastically changes the release of copper from the matrix. The ion release rate from nanocomposites increased quickly exhibiting a sharp maximum during the first day; meanwhile, in microcomposites, the release rate increased slowly releasing lower ions. The relevance of particle size was confirmed by the antibacterial behavior of the samples as polypropylene with nanoparticles displayed larger activities against *Staphylococcus aureus* and *Pseudomonas aeruginosa* bacteria than microcomposites. These results further showed the relationship between copper ion release and antimicrobial behavior in polymer/metal composites. Our findings further revealed that the ion release from polymer composites could be improved by either increasing the hydrophilic characteristic of the matrix or by lowering its crystallinity. These observations allowed the conclusion that both the polymeric matrix and the size of the metal filler are relevant variables toward the design of antimicrobial composite materials.

Keywords

Controlled release, metal ions, nanomaterials, nanocomposites, microcomposites, antimicrobial materials

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Introduction

Copper can be considered as an outstanding material for the development of antimicrobial materials. During the last years, its alloys, salts, and oxides have been recognized as strong biocide materials against a broad range of bacteria.^{1,2} Today, copper is used as a water purifier, algacide, fungicide, nematocide, molluscicide, and antibacterial and antifouling agent.^{1,3} Although this knowledge was originally based on empirical evidences, today several scientific results support the strong antimicrobial behavior of copper. Wilks et al.,⁴ for instance, studied a set of metallic surfaces showing that those containing copper are the most effective in reducing the survival of bacteria. All this knowledge further allows the design of more complex copper-based antimicrobial materials such as nanoparticles or novel multifunctional biomaterials.^{2,5–10}

The mechanism of copper toxicity can be explained by several processes such as displacement of essential metals from their native binding sites or through ligand interactions.¹ Although Cu^+ contributes to the toxicity producing highly reactive hydroxyl radicals by Fenton-like reactions,¹¹ Cu^{2+} can be considered as the main biocide agent.¹² Cu^{2+} is able to form organic complexes with functional groups present in the microorganisms resulting in either defects in the conformational structure of nucleic acids and proteins or changes in the oxidative phosphorylation reactions and in the osmotic balance. Copper ions are therefore the real active compounds either killing (cidal effect) or inhibiting the growth (static effect) of microorganisms.¹

Regarding the influence of the microorganism on the biocide behavior of copper, Gram-positive bacteria are more affected by this metal than Gram-negative bacteria as the latter present a double membrane and the periplasmic space decreasing the ions transport.^{2,13,14} However, the toxic mechanisms reported for copper are the same for both types of bacteria. Copper has also been used for centuries as a fungicide agent although showing less toxicity as compared with prokaryote microorganisms because of its different sizes and structures.^{2,15} In this case, the toxicity has other mechanisms such as metal trapping by cell-wall components, altered uptake of copper, extracellular chelation, precipitation by secreted metabolites, and intracellular complexing.¹⁶

It is noteworthy that the strong antimicrobial effect of copper can be rendered to different matrices such as polymers or ceramics by adding its ions or particles.^{10,15,17,18} Regarding polymers, although different kinds of particles can be embedded in these matrices, nanometric fillers are considered good candidates due to their outstanding properties. For instance, Damm et al.¹⁹ demonstrated that silver nanoparticles in polyamide matrices presented higher ion release rates than microparticles due to their extremely high specific surface area. Copper nanoparticles (NPCu) have been mixed with different polymer matrices, such as polyvinylmethyl ketone (PVMK), polyvinylchloride (PVC), and polyvinylidene fluoride (PVDF), displaying antifungal and bacteriostatic behavior.¹⁵ This approach further allowed the development of novel polymer coatings with potential biocidal behavior.²⁰ Our group further showed that polypropylene (PP)/NPCu composites prepared by melt blending presented tailored antimicrobial behavior depending on the kind of NPCu and their concentration.^{17,18} The toxic mechanism of these polymer/NPCu composites is based on their ion release ability as a direct relationship between this property and the antimicrobial behavior was concluded. A similar tendency was found in polymer/silver composites.²¹ The polymer matrix is therefore a critical factor in the biocide behavior of the composites as independent of the metal filler (silver or copper); the ion release rate further depends on the matrix properties.^{15,21}

Based on the hypothesis that the ion release is the main mechanism explaining the antimicrobial behavior of polymer/metal composites, the goal of this article is to analyze the effect of two variables on this property: particle size and matrix properties. We focus on the particular case of copper-based composites. Our results show that by changing these variables, the copper release rate can be changed improving the antimicrobial behavior of the composites.

Experimental section

Materials and sample preparation

The main polymer matrix was a commercial-grade isotactic PP with a melt index of 3.3 (g/10 min) (2.16 kg/190°C) from Petroquim S.A. (Chile). The polyamide 6 (PA6) and the high-density polyethylene (HDPE) are also commercial grade provided by a petrochemical company. The propylene-co-1-octadecene copolymer was synthesized by a metallocenic catalytic system having around 8 mol% of comonomer content. The copolymerization was carried out for 30 min under inert nitrogen atmosphere using a 1-L glass autoclave reactor with toluene as a solvent. The reactor was equipped with a temperature and internal pressure control system and with stirring. The catalyst used was the *rac*-Me₂Si(2-Me-Ind)₂ZrCl₂ (Boulder Scientific Company, Mead, Colorado, USA), and the cocatalyst was methylaluminoxane (Chemtura Manufacturing UK Limited, Manchester, UK), 1-Octadecene (Sigma–Aldrich, St. Louis, Missouri, USA) was distilled and added to the reactor to complete with the solvent a total volume of 0.5 L. The reactions were made at 5×10^{-6} moles of catalyst, 45°C, Al/Zr ratio = 1000, and a monomer pressure of 2 bar. Details can be found elsewhere.²²

The copper nanoparticles (NPCu) with an average particle size of 10 nm as measured by high-resolution transmission electron microscopy (TEM) were supplied by Versus Co. Ltd (Chile). The copper microparticles (MPCu) with an average particle size of 45 μm were provided by Fluka Co. Ltd (St. Louis, Missouri, United States). These particles were analyzed by wide-angle X-ray diffraction by using a Siemens D-5000 diffractometer with CuKα = 1.54 Å and a step scan of 0.02° at room temperature.

The composites were prepared using a Brabender Plasticorder batch system (melt volume of 35 cm³) at a temperature 30°C higher than the melting point of the polymer matrix, 110 r/min and 10 min, under a nitrogen atmosphere to avoid oxidative degradation processes. The composites with 1 and 5 vol.% of filler were prepared. The composites were press molded at a temperature 30°C higher than the melting point of the matrix and at 50 bar for 5 min and cooled under pressure by flushing the press with cold water, producing a film about 1 mm thick. For the different trials, the samples were cut to obtain 20 mm × 30 mm pieces.

Antimicrobial tests

The antimicrobial test used was based on the international standard for plastic surfaces (ISO 22196). Similar methods have been widely applied to test the antimicrobial activity of copper alloys.⁴ Two representative bacteria were studied: methicillin-resistant *Staphylococcus aureus* (ATCC 433000) and *Pseudomonas aeruginosa* (ATCC 27853). This selection is based on the fact that these bacteria can explain most of the infections found in medical devices.²³ The bacteria were grown overnight in Luria broth at 37°C. The bacterial count was then standardized to 5 log₁₀ colony forming unit (CFU)/mL by measuring their optical density at 600 nm, and they were kept at 37°C for 2 h to avoid the lag phase of the kinetic bacterial growth. The initial concentration of bacteria was determined by the solid agar plate method. An aliquot of 50 μL of the bacterial broth was poured into the sample surface and covered with a glass during a specific time ranging from 0, 30, to 90 min 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 diluting the concentration by 0.01. From this dilution, a 40-μL aliquot was extracted, transferred onto nutrient agar, and incubated for 16 h at 37°C before counting the colonies. To determinate the total numbers of bacteria in the original solution, the measurements were corrected by the dilution factor by

dividing the numbers of CFUs by the product of the dilution factor and the volume of the plated diluted suspension. This value was the number of bacteria per milliliter presented in the original solution. All the experiments were repeated at least three times on different days. The samples, tools, glass materials, and wells were sterilized in an Orthmann autoclave for 20 min at 1 bar and 140°C before the experiment.

Copper ion release

The release of cupric ion (Cu^{2+}) was measured using an ultraviolet (UV)–visible (Model JENWAY 6320D) spectrophotometer based on the method reported elsewhere for copper alloys and copper nano- and microparticles.²⁴ 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 and a calibration of absorbance versus cupric ion was obtained by measuring the chelate solution of various CuSO_4 concentrations. The amount of cupric ion dissolved in the sample solution was calculated from the measured absorbance that is proportional to the amount of cupric ions.²⁴

Ions released from copper particles were determined by adding 0.05 g in 40 mL of deionized water. Before each measurement at time, the solution was centrifuged for 15 min at 19,000 r/min and 6 mL was extracted for the measurements. Then, the extracted 6 mL was replaced for 6 mL of deionized fresh water. For the measurement of ion released from the composites, the samples of 20 mm × 30 mm and 1 mm thick were used. The plates were immersed in 40 mL of deionized water. After each measurement, all the deionized water was replaced for deionized fresh water. All the experiments were repeated at least three times.

Sample characterization

The mechanical properties were measured with an HP D500 dynamometer (HP Industry, Buenos Aires, Argentina) at a rate of 50 mm/min at 23°C and 30% relative humidity. The melt temperature (T_p) and melt enthalpy (ΔH_f) of the polymer matrices were measured by differential scanning calorimetry (DSC) on a TA Instruments Q200 in an atmosphere of nitrogen. The sample mass varied between 5 and 10 mg. Each sample was subjected to a heating of 20°C/min from room temperature to 20°C above the melting temperature and then cooled at the same rate to 25°C. The crystallinities of the samples were evaluated from the ratio between the melt enthalpy of the sample and the melt enthalpy of a perfect crystalline material (ΔH_f°) by

$$\text{Crystallinity} = \left(\frac{\Delta H_f}{\Delta H_f^\circ} \right) \times 100\%$$

Results and discussions

Characterization of the particles

In order to understand the processes involved in the ion release from polymer/copper composites, the effect of size was first analyzed for pure particles. Figure 1 shows the effect of time on both the metal ion release rate (left y-axis) and the ions released (right y-axis) from NPCu and

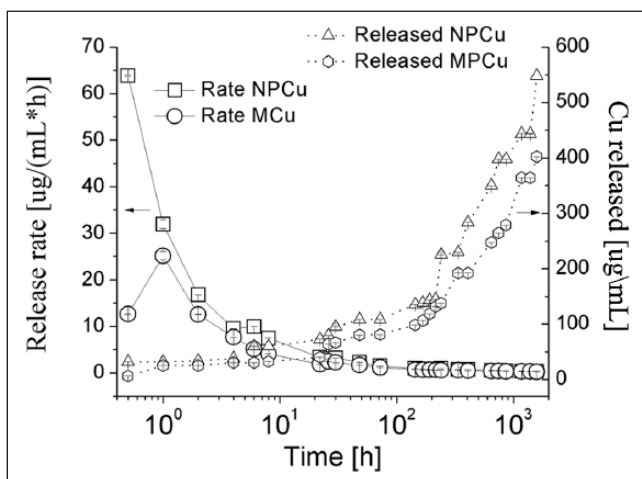


Figure 1. Effect of time on the copper ion release rate (left y-axis) and on the concentration of copper ions released (right y-axis) from copper nanoparticles (NPCu) and microparticles (MPCu) soaked in deionized water.

MPCu immersed in water under the same conditions. Microparticles displayed a smooth maximum in the release rate at short times (~1 h); meanwhile, nanoparticles presented a sharp maximum at shorter times. At longer times, both particles had similar release rates and relevant differences were not observed. The copper ions released (right y-axis) complemented this tendency by showing larger ion concentrations in the medium with NPCu than with MPCu. From Figure 1, it is concluded that the strong effect at short corrosion times of the particle size influences the ion release whereas at longer times differences between both particles were not found. A similar tendency was reported by Xia et al.²⁴ associated with both the larger specific surface area and the higher surface energy of NPCu as compared with microparticles. The interaction between copper and water is facilitated in nanoparticles because of their high surface energy as reported by these authors. Moreover, a CuO film was observed in microparticles retarding the corrosion processes, whereas in nanoparticles an easily dissolved Cu₂O layer was formed explaining the behavior found.^{18,24}

To confirm these results, the pH of the solutions containing copper particles was tested after 70 days of immersion in 40 mL of deionized water at 25°C. The pH value at the beginning of the experiment was 5.51. MPCu increased the pH value to 6.10, whereas NPCu increased the pH to 6.28. The pH increment can be associated with OH⁻ ions generated by the formation of cupric ions from copper particles. These results further confirm the effect of particle size on the ion release processes.

Figure 2 displays the X-ray diffraction patterns from both particles before and after immersion during 100 days in water. Before immersion, both particles displayed diffraction peaks at 43.2°, 50.2°, and 74.2°, meaning the presence of pure copper crystals. After immersion, the same diffraction peaks were mainly observed in both particles although new peaks emerged. Immersed MPCu presented a new broad peak at 36.6° with low intensity meaning the formation of a layer of Cu₂O crystals. NPCu otherwise displayed the same new crystal structure although more defined as concluded by the narrower peak at 36.6° and the new peak at 42.2°. These results showed that the ion releases observed in Figure 1 can be associated with corrosion processes.

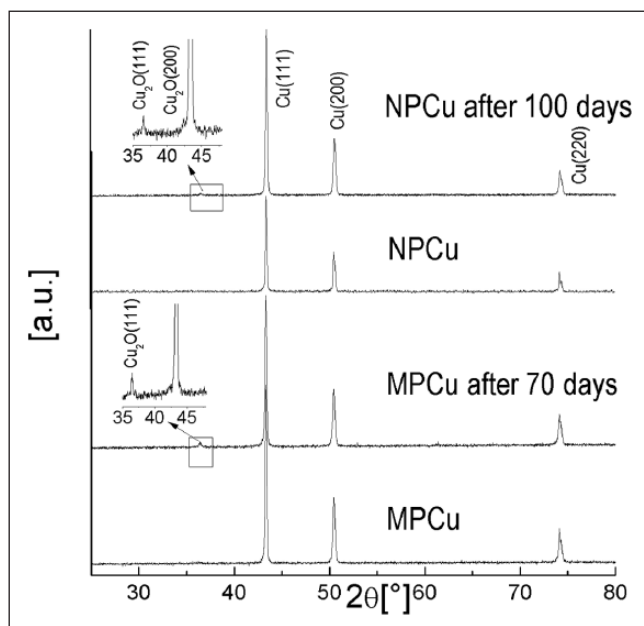


Figure 2. X-ray diffraction of NPCu and MPCu before and after the immersion in deionized water by 100 days.

Characterization of polymer/copper composites

Figure 3 (left y -axis) show the metal ion release rate from composites of PP with 1 and 5 vol.% of both MPCu and NPCu particles, and the associated ion concentration in the medium, respectively. Similar to pure particles, the filler size was relevant in the ion release as nanocomposites presented larger rates and released more ions than microcomposites. The particle size further modified the effect of the filler concentration on the ion release from the composites. When the nanoparticle concentration was increased in the polymer, the release of metal ions was also increased; meanwhile, for micrometric fillers, their concentration in the polymer barely changed the copper release. Indeed, the microcomposite with 5 vol.% of filler displayed lower ion releases than nanocomposites with 1 vol.%. As time elapsed, the difference between nanocomposites and microcomposites increased independent of the amount of filler, whereas in the pure particles a steady difference between both particles was mainly observed (see Figure 1).

The maximum in the release rate observed in pure nanoparticles was still observed in nanocomposites, independent of the amount of filler. In microcomposites, otherwise this maximum was not displayed (see Figure 1). The tendency in nanocomposites is quite relevant as in polymer-based drug delivery systems this kind of maximum is currently associated with a “burst” effect from the particles or molecules on the surface of the material. However, X-ray photoelectron spectroscopy (XPS) analysis showed that NPCu were not presented on the surface of similar samples.¹⁷ Moreover, the maximum was still observed in composites immersed after an experiment of 100 days of immersion.¹⁸ Therefore, the maximum observed in these nanocomposites comes from an intrinsic phenomenon of the particles as displayed in Figure 1. Although the maximum in the release rate was observed in nanocomposites, it was shifted to larger times meaning new processes in the ion release due to the polymeric matrix (see below for details). Noteworthy, a larger release of metal

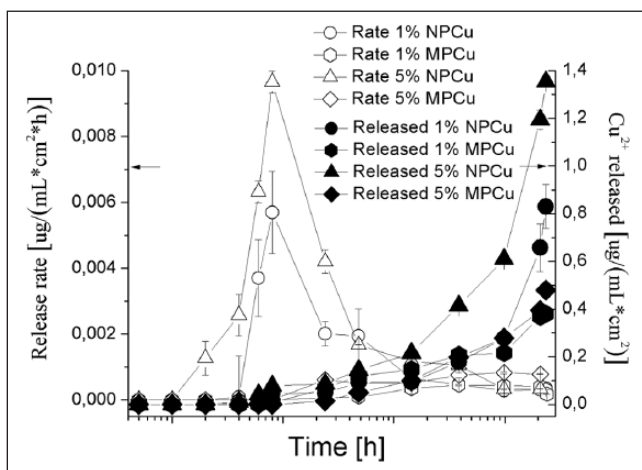


Figure 3. Effect of time on the copper ion release rate (left y-axis) and on the concentration of copper ion released (right y-axis) from polypropylene composites having 1 and 5 vol.% of copper nanoparticles (NPCu) and microparticles (MPCu).

ions was observed in nanocomposites than in microcomposites (Figure 3, right y-axis) following the tendency observed in pure particles. Therefore, nanoparticles in polymer matrices released more ions than microparticles because the particles itself showed this behavior. Similar results were found in polymer/silver systems although without a comparison between the polymer composites and the pure particles.¹⁹

To quantify the effect of the polymer matrix on the metal ion release from the particles, the release values were normalized by the total amount of particles used (a conversion parameter). Therefore, the ratio between the ion release and the grams of copper presented in the experiment was measured. In this way, we found that the release of ions from copper particles was reduced 10 times when the polymer was present from roughly 100 to 10 mg/L (g of copper). Therefore, the polymer matrix produced both a reduction in the ion conversion and a shift to longer times in the maximum release rate, showing evidences about the ion release from polymer composites. In particular, our results confirmed the relevance of water and oxygen diffusion through the polymer bulk, both interacting with the surface of the embedded particles by the standard corrosion reactions. However, water should diffuse through the polymer to reach the particles (diffusion-controlled system) retarding the whole process and shifting to longer times the maximum in the release rate (Figures 1 and 3). Although PP is highly non-polar, water molecules can diffuse through its interconnected amorphous parts defining a percolated network.²⁵ Similar results have been published in polymer/silver systems.²¹ Therefore, water diffuses through the composite forming first a Cu_2O layer on the surface of NPCu that can be easily converted into Cu^{2+} because of its high reactivity.^{18,24,26} The reduction in the copper ion release by a factor of 10 (normalized values) when the polymer was presented can also be explained by this diffusion-controlled hypothesis as the amount of water reaching the particles could not be enough to fully develop the corrosion processes. It is also likely that water does not reach all the particles explaining also the drastic decrease in ion release. However, the last hypothesis is not able to explain the shifting in the maximum observed in the release rate of composites. Therefore, both hypothesis (polymer both limiting water diffusion and “hidden” part of the particles) could together explain the experimental findings.

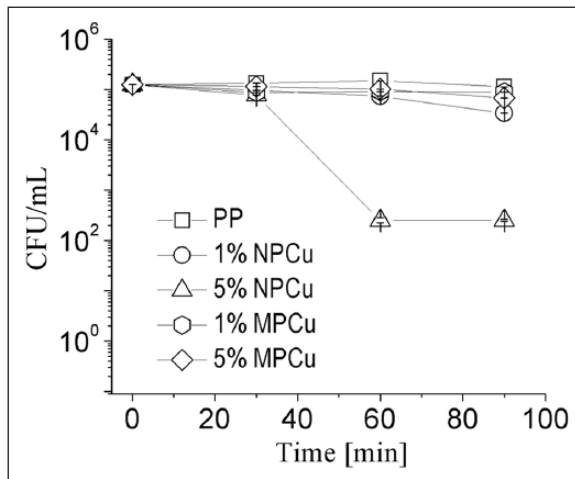


Figure 4. Effect of time on the antimicrobial activity against *Staphylococcus aureus* of different polypropylene/copper composites.

Antimicrobial characterization

The mechanisms of copper toxicity toward microorganisms are associated with several processes such as presence of radicals, displacement of essential metals from their native binding sites, and ligand interactions. From the material point of view, we can state that independent of the specific mechanism, the presence of copper ions is a key element. Therefore, any material able to release copper ions above some threshold will display some antimicrobial activity. In particular, higher antimicrobial behavior will be found in materials with higher ion release. This hypothesis explains the results from copper metal and its alloys showing that the antimicrobial activity of these materials increased with time and with the temperature.^{3,27} It also explains why the antimicrobial efficiency in alloys increases with the copper content where the largest activities were found in pure copper metal surfaces because of their high ion release as compared with alloys.⁴ In polymer/copper and polymer/silver composites exist a similar tendency relating amount of the particle in the polymer with both the metal release and the antimicrobial activity.^{17,18,21} Therefore, the antimicrobial effect of copper particles embedded in polymer composites should be related to the ability to release metal ions. Based on both this hypothesis and Figure 3, composites based on NPCu will present higher antimicrobial behavior than those based on MPCu.

To discuss the relationship between ion release and antimicrobial behavior in our case, it should be realized before that the ion release tests were carried out in deionized water during long times (up to 70 days), whereas the antimicrobial tests were done in a medium culture during short times (up to 90 min). Indeed, the antimicrobial behavior of the composites depends on their ability to release ions in the bacteria medium and more specifically in the bacteria surface region. The dissolution process of copper is highly affected by the different components present in the culture media, such as organic components including the bacteria (microbial induced leaching).^{28,29} For instance, bacterial membranes contain macromolecules with highly electronegative chemical groups serving as sites for adsorption of metal ions and therefore changing their effective concentration.³⁰ In this context, the real concentration facing the bacteria can not be measured and our analysis was based on the the global ion release behavior of the samples in deionized water .

Figures 4 and 5 show the antimicrobial activity of PP composites against *S. aureus* and *P. aeruginosa*, respectively. The initial concentration of bacteria was $1 \pm 0.5 \times 10^5$ CFU/mL and the

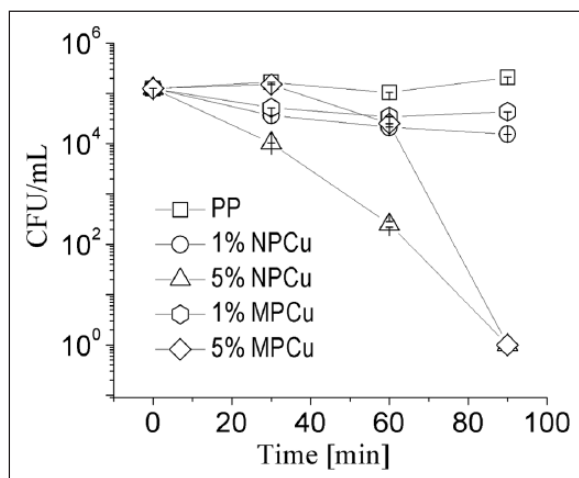


Figure 5. Effect of time on the antimicrobial activity against *Pseudomonas aeruginosa* of different polypropylene/copper composites.

survival was tested after 30, 60, and 90 min of contact. Bacterial concentration of both pathogens on the control sample (PP without copper particles) was not affected even after 90 min of exposition. The addition of copper particles into PP otherwise rendered strong antibacterial effect to the matrix and this activity was stronger using nanoparticles. By increasing the contact time between the bacteria and the sample, the antimicrobial efficiency was raised as reported previously in copper alloys⁴ and polymer/copper composites.¹⁸ This tendency confirmed the relationship between the ion release from the sample and its antimicrobial activity. The reason is that longer contact times imply higher ion release from the sample affecting more the bacterial viability (Figure 3).

PP/NPCu composites displayed larger activities against both bacteria than PP/MPCu, especially at times lower than 90 min. In particular, PP/NPCu composites containing 5 vol.% of copper was able to reduce the concentration of *S. aureus* in 99.8% after 60 min, while PP/MPCu showed a much less antimicrobial activity at this time. For *P. aeruginosa*, the same tendency was observed confirming the effect of particle size. The antimicrobial behavior, however, depended on the bacteria as larger antimicrobial effects were observed in *P. aeruginosa* than in *S. aureus*, independent of the particle used.

A relevant variable affecting our discussion about the antimicrobial behavior of the composites is their surface characteristics as reported elsewhere.^{28,29} In our case, any surface change should come from the presence of copper particles as the composites were based on the same matrix. In a previous article, the amount of copper near the sample surface, either as ions or as nanoparticles, was studied in similar samples by XPS.¹⁷ Despite the high amount of copper present in these composites, their surface did not present any relevant evidence of copper.¹⁷ Even after sputtering the surface with argon ions, the signal associated with copper did not change significantly, showing that nanoparticles were covered by a polymer film of several hundred of nanometers. These results showed that the surface of our composites was unaffected by the particles and therefore was not included in the discussion about the antimicrobial behavior of the composites.

Regarding the biocide mechanisms of our antimicrobial materials, we should remember that the toxicity arising from the mixture of an antimicrobial agent into a non-active polymer is as similar as the toxicity from the agent itself.^{31,32} In biocide copper surfaces, the exact biocide mechanisms and their details remain issues of debate although it is well known that bacteria suffer rapid membrane damage and DNA degradation, in addition to other less defined mechanisms.³³ In addition, the

sequence of events may depend on the type of microorganism. However, quantitative proteomic profiling supports the membrane damage as a key event.³⁴ In this way, bacteria initially suffer a severe damage of the cell envelope upon contact with an antimicrobial metal surface allowing the access of copper ions to cellular components, where further damage ensues.³³ Independent of the exact mechanisms, at least two conditions are needed for a metal to be antimicrobial:³³ (1) to have a redox-active surface under ambient conditions and (2) to release ions toxic to cells. The first condition relates with the hypothesis that bacterial metal contact permeabilizes the cells facilitating access of copper ions to cellular components, whereas the second condition relates with the fact that copper ions released from the surface play also a crucial role. The fact that antimicrobial polymer/metal composites do not show particles on their surface implies that the second condition related to the release of ions is the main mechanism for toxicity in our samples.¹⁷

In polymer/NPCu composites, new processes could appear associated with the metal nanoparticles. In this case, the proposed mechanisms of toxicity can be separated into two depending on the species considered as the active agent: (1) the nanoparticle or (2) the metal ions released from the particles.³⁵ The first mechanism is based on the “Trojan-horse” effect meaning that copper nanoparticles penetrate to the cell wall releasing inside their ions.^{35,36} However, a growing number of reports indicates that the ion release is the driving force for the antimicrobial behavior of metal nanoparticles.^{27,37} This hypothesis is supported by recent results from copper oxide nanoparticles showing the absence of the “Trojan-horse” effect.³⁵ Indeed, most of the analyses regarding antimicrobial metal nanoparticles focused on the metal ion release instead of the particles absorbed by bacteria.^{5,38,39} This was confirmed by the results coming from polymer/metal nanocomposites where the antimicrobial effect of these materials related to the metal ion release rather than with the leaching of the particle.^{17,18} The presence of a polymer film covering the particles as found previously by XPS analysis in polymer/copper composites further ruled out the direct contact between the bacteria and the copper particles.¹⁷ This is confirmed by analyzing the diffusion coefficient of metal nanoparticles in thermoplastic polymer matrix that can be calculated by the Stokes–Einstein relation.⁴⁰ By using the Williams–Landel–Ferry equation for the value of the dynamic viscosity, the authors estimated the diffusion coefficient of a PP to be around $1.07 \times 10^{-19} \text{ m}^2/\text{s}$ at 25°C. With this value, any significant migration of metal nanoparticles can be expected at long times solely in the case of particles with radius in the order of 1 nm.⁴⁰ Moreover, these models do not take into account the crystal phase of the polymer matrix or the agglomerations of nanoparticles. Therefore, the ion release from polymer/NPCu is the main toxic mechanism.

Effect of polymer matrix on the copper ion release

The results regarding the relevance of metal ions on the toxic mechanism of polymer/copper composites motivated us to further analyze the effect of the polymer matrix on these processes. Our hypothesis states that water diffusion through the amorphous phase of PP is crucial for the corrosion of copper, and therefore, less crystalline matrices should release more ions. Moreover, by increasing the polarity or hydrophilic characteristic of the matrix, more water should be absorbed into the polymer increasing the release of copper ions. To support our hypothesis, a set of polymers having different characteristics, as displayed in Table 1, were melt mixed with 5 vol.% of NPCu and their ion release was measured. Figure 6 shows the relevance of the polymer matrix on the release of copper ions with the largest values obtained with the PA6 matrix having the highest hydrophilic behavior. PA6 has high tendency to react with water increasing the diffusion process and facilitating the mass transport. Moreover, in these polymers, water can change the overall crystalline state acting as a plasticizer agent increasing the chain mobility.^{20,41,42,46} The lowest ion releases were obtained with the HDPE

Table 1. Main characteristics of different polymer matrices used for composites with 5 vol.% of copper nanoparticles.

Sample	Hydrophilic level	Contact angle H ₂ O (°)	Crystallinity (%)	Tensile elastic modulus (MPa)
PP	Low	116.0 ^a	43 ^b	1100
PPC18	Low	N/A ^c	20 ^b	50
HDPE	Low	101.7 ^d	65 ^e	900
PA 6	High	61 ^f	25 ^g	1800

PP: polypropylene; PPC18: propylene-1-co-octadecene copolymer; HDPE: high-density polyethylene; PA 6: polyamide 6. Crystallinity values are measured from differential scanning calorimetry.

^aPolymer data handbook, Edt. J. Mark, 1999, Oxford University Press, Inc.

^bValue for perfect crystalline PP 209J/g. Polymer data handbook, Edt. J. Mark, 1999, Oxford University Press, Inc.

^cValue not found. It can be assumed to be similar to the values from PP and HDPE.

^dN. De Geyter, R. Morent, C. Leys. *Surf. Interface Anal.* 40 (2008); 608–611.

^eValue for perfect crystalline HDPE 290J/g. S. Sinha Ray, M. Biswas. *Adv. Polym. Sci* 155(2001); 167–221.

^fR. Misra, B.X. Fu, A. Plagge, S. Morgan. *J. Polym. Sci. Part B* 47 (2009); 1088–1102.

^gValue for perfect crystalline PA6 290J/g. M. Smita, K.N. Sanjay. *Polym. Comp.* 28 (2007); 153–162.

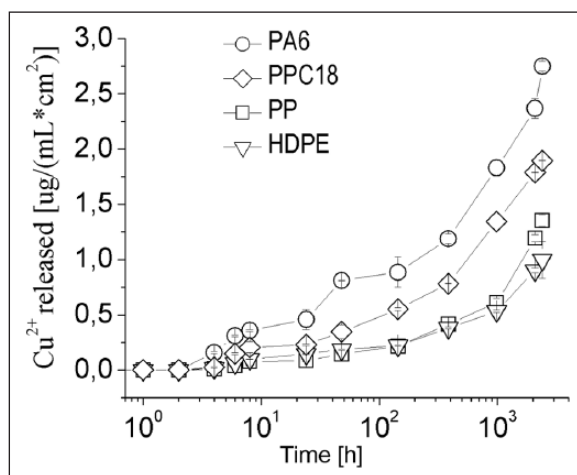


Figure 6. Effect of time on the concentration of copper ion released from composites based on matrices of different characteristics (see Table 1) having 5 vol.% of copper nanoparticles (NPCu) and microparticles (MPCu).

matrix having both low hydrophilic level and high crystallinity. The effect of the crystallinity is further confirmed by comparing the behavior of PP with its copolymer (propylene-1-co-octadecene copolymer (PPC18)), both having barely the same polarity but large differences in crystallinity. Low crystalline PPC18 displayed ion releases 50% larger than high crystalline PP. To preliminarily analyze the effect of the polymer matrix on the antimicrobial behavior, a comparative test for HDPE and PP composites was performed. As expected, HDPE composites, having the lowest ion release, showed lower antimicrobial reduction (88%) than PP composites (94%) although these results were not statistically significant. It seems that in this case, the difference in the ion release between both matrices was not enough to see an effect on the antimicrobial behavior. However, this result showed that two different matrices having

Table 2. Effect of the addition of copper particles on the mechanical behavior of polypropylene.

Filler content (vol.%)	Particle	Elastic moduli (MPa)	Yield stress (MPa)
0	N/A	1089 ± 22	28.3
1	MPCu	1022 ± 37	28.2
5	MPCu	1290 ± 42	28.4
1	NPCu	1265 ± 95	31.2
5	NPCu	1292 ± 36	29.6

N/A: not available; MPCu: copper metal microparticles; NPCu: copper nanoparticles.

ion releases of the same order of magnitude can display similar antimicrobial behavior confirming the relevance of the copper release.

The relevance of water diffusion and polymer microstructure has been reported for polyamide/silver systems where the dual sorption theory was proposed.^{42,43} This theory is based on two different physical mechanisms that affect mass transfer: (1) diffusion that is a very fast process and (2) embedding that is a low process related to the intermolecular forces between the penetrant molecules and the polymer specimen. On the other hand, the composites displayed similar behaviors at short times presenting a maximum in the release rate similar to that observed in Figure 3 (results not shown). Even highly non-polar matrices such as PP and HDPE, where interactions between water and polymer chains are low, presented this maximum. This confirms our hypothesis that this peak is related to the behavior of the particles itself.

A variable that should be considered in our analysis is the mechanical property of the matrix.^{44,45} By increasing the elastic modulus of the polymer matrix, a reduction in the diffusion coefficient can be obtained.⁴⁴ The tensile elastic moduli of the different matrices used are displayed in Table 1. In our case, the sample with the highest stiffness presented the highest release of metal ions meaning that the positive effect of polarity in PA was more relevant than the negative effect of its high elastic modulus. However, the effect of water absorption during the test, decreasing its mechanical properties, should be considered.⁴⁴ In non-polar samples, the mechanical behavior was more relevant and it could explain, at least partially, the high ion release of PPC18 having the lowest elastic modulus (PPC18). In these samples, the mechanical behavior is inversely proportional to the crystallinity.^{45,47} Therefore, PPC18 presented higher ion release than PP and PE because of its low crystallinity implying (1) larger interconnected amorphous parts²⁵ and (2) low mechanical stiffness.⁴⁴ Moreover, changes in the free volume associated with the branches presented in PPC18 can facilitate the ion diffusion.⁴⁸

Regarding the effect of the particles on the mechanical behavior of the matrix, Table 2 shows that composites presented a slight increase as compared with the pure matrix. With 1 vol.% of nanoparticles, the elastic modulus increased around 20%, whereas microparticles at the same concentration did not modify this property. At 5 vol.% of filler, both particles enhanced the elastic modulus around 20%. The higher agglomeration in composites with high amount of nanofiller, as reported in PP composites with silica nanoparticles, explains the tendency in NPCu.⁴⁹ However, changes in the mechanical behavior of composites are not associated with changes in the polymer but rather with the contribution of the filler depending on its properties and interactions with the matrix.⁵⁰ Indeed, mechanical theories based on continuum models assume that the property of the matrix does not change with the incorporation of the particle.⁴⁹ Nanoparticles can modify the mechanical behavior of polymer molecules present in the proximity of the particle surface, but it is not relevant at these concentrations.^{49,50} Based on this, we assume that changes in the mechanical behavior of the composites did not affect the release process.

Regarding the potential application of these antimicrobial PP/copper composites, it is stressed the steadily growth of this polymer in healthcare applications. This growth is associated with the PP properties such as low chemical reactivity, high radiation resistance, high stiffness, high clarity, high barrier behavior for gases and liquids, high impact, high flexibility, and low cost. PP products in this field include several medical devices, packaging products, and delivery systems for solid and liquid pharmaceuticals.⁵¹ It is estimated that about a quarter of disposable and a third of non-disposable medical devices are PP-based, while global growth rate for PP use in healthcare is about 8.7% per year.⁵² In particular, suitable future applications can be related to hospital products with high probability to be infected such as bed guardrails, tray tables, door knobs, carts, and portable poles that hold intravenous bags.

Conclusion

The size of copper particles was a relevant parameter in the release mechanisms of metal ions. The ion release rate from nanoparticles presented a sharp maximum having as consequence larger ion releases than microparticles. Noteworthy, this tendency was also displayed in polymer composites as nanocomposites displayed larger ion release than microcomposites. These results explained the stronger antimicrobial behavior of polymer nanocomposites against *S. aureus* and *P. aeruginosa* as compared with microcomposites. Our results showed that the processes involved in the ion release of the composites, and therefore in their antimicrobial behavior, can be associated with water diffusion through the polymer. This hypothesis was confirmed observing the strong effect of the polymer characteristics on the release of copper ions. In particular, either hydrophilic or low crystalline matrices favored the copper ion release. Based on these findings, a proper design of antimicrobial polymer/copper composites can be carried out by controlling polymer characteristics and particle size.

Funding

The authors gratefully acknowledge the financial support of CONICYT, project FONDECYT 1110078, and Office of Naval Research Global, Department of the Navy, US, Project N62909-11-1-7066 ACO: N62927 ONRG LTR 7066.

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