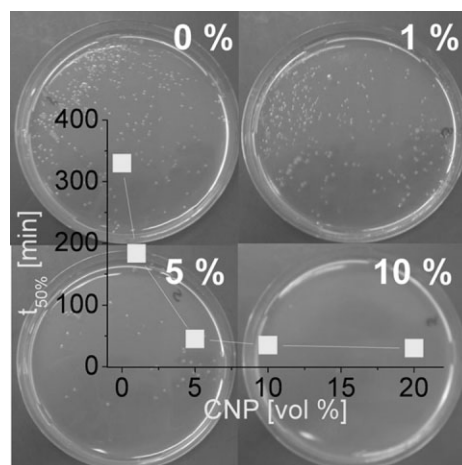


# Toward Tailor-Made Biocide Materials Based on Poly(propylene)/Copper Nanoparticles

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A set of poly(propylene) composites containing different amounts of copper nanoparticles (CNP) were prepared by the melt mixed method and their antimicrobial behavior was quantitatively studied. The time needed to reduce the bacteria to 50% dropped to half with only 1 v/v % of CNP, compared to the polymer without CNP. After 4 h, this composite killed more than 99.9% of the bacteria. The biocide kinetics can be controlled by the nanofiller content; composites with CNP concentrations higher than 10 v/v % eliminated 99% of the bacteria in less than 2 h. X-ray photoelectron spectroscopy did not detect CNP at the surface, therefore the biocide behavior was attributed to copper in the bulk of the composite.



## Introduction

Bacterial contamination of medical devices is a large complication leading to significant morbidity and mortality,<sup>[1]</sup> such as, for example, foodborne infections.<sup>[2]</sup>

In particular, it is the main cause of implant failure in modern medical disciplines using biomaterials.<sup>[3]</sup> Actually, verocytotoxicogenic *Escherichia coli* infections cost the United States almost US\$ 1 billion a year in medical costs and lost of productivity.<sup>[2]</sup> Therefore, a high demand for new materials able to reduce the above mentioned infections is expected, in particular in the area of polymer science.

Biocidal polymers can be prepared by the incorporation of a biocide agent into bulk polymers during processing of the material or by endowing a biocidal function to the polymer after processing, for example, applying surface coatings containing the agent.<sup>[3–6]</sup> A different approach is the polymerization of monomer-containing biocide groups or its copolymerization with another monomer producing a new family of polymers bearing biocidal agents.<sup>[3]</sup> The grafting of antimicrobial agents into the polymers is yet another approach to prepare bioactive materials.<sup>[3]</sup> A new route based on the polymerization of the biocide polymer on the surface of commercial polymers by atom transfer radical polymerization has recently been reported.<sup>[7,8]</sup> Of

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all the above mentioned methods, the direct addition of the biocide agent into the bulk polymer seems to be the most efficient and simplest route, as it can be easily implemented in standard polymer processing units without any kind of post-reactor chemical reactions on the polymer. After the article from Damm et al. demonstrating that silver metallic nanoparticles in polyamide matrices present a higher ion release rate than microparticles, due to their extremely high specific surface area,<sup>[9]</sup> the use of polymeric composites based on metallic nanoparticles for biocidal applications has been highlighted. Silver based hybrid materials are one of the most studied agents for preparing composites in this area because of their strong and versatile antimicrobial properties and low toxicity.<sup>[10–15]</sup> Antimicrobial properties of polymeric composites with silver nanoparticles have been reported using polyamide,<sup>[9,13]</sup> poly(propylene),<sup>[16]</sup> and poly(acrylate),<sup>[17]</sup> among other polymeric matrices.<sup>[17,18]</sup> Nevertheless, despite the well known biocide properties of copper, as shown recently,<sup>[2,19–21]</sup> the study of copper based biocidal polymeric materials has been less extensive, particularly regarding copper nanoparticles (CNP). Copper is essential to human health since it is involved in normal metabolic processes and any excess can be eliminated from the human body. Therefore, copper is considered a safe metallic compound by some authors (see, for example, ref.<sup>[21]</sup>).

Polymer/CNP composites with antifungal and bacteriostatic properties using polyvinylmethyl ketone, poly(vinyl chloride), and polyvinylidene fluoride as polymeric matrices have been reported.<sup>[22]</sup> Polyethylene/CNP composites for intrauterine devices have also been developed, exhibiting excellent bioactive properties.<sup>[23,24]</sup> Another approach to preparing biocide hybrid-materials based on CNP has been the incorporation of particles at the surface of polymers. Copper ion implantation by plasma immersion was utilized to produce an antibacterial surface on polyethylenes.<sup>[25]</sup> A relatively large amount of copper, about 11%, was implanted into the near surface region by this method. All the samples presented excellent antibacterial properties.

Despite the above mentioned articles concerning the preparation of biocide polymeric composites, a deep characterization of some relevant parameters, such as the filler content, is still needed to optimize the antimicrobial behavior of the new materials. In this context, the main goal of the present contribution is to quantify the effect of the CNP content on the antimicrobial behavior of poly(propylene) composites prepared by the melt mixed method and to analyze the effect of the surface properties on the biocidal mechanism. Our results will open up new tailor-made polymeric biocide materials based on copper nanoparticles with relevant biomedical applications, among others.

## Experimental Part

### Materials

The polymeric matrix was a commercial grade isotactic poly(propylene) (PP) with a melt index of 3.3 g/10 min from PETROQUIM (Chile). The copper nanoparticles (CNP) with an average particle size of 5 nm, as measured by high resolution transmission electron microscopy (TEM), were supplied by Versus Productos Industriales S.A. (Chile). The *Escherichia coli* strain used to test the antimicrobial behavior was a DH5 $\alpha$ .<sup>[26]</sup>

### Preparation of PP/CNP Composites

The composites were prepared using a Brabender plasticorder at 190 °C, 110 RPM for 10 min, under a nitrogen atmosphere to avoid oxidative degradation processes. CNP composites with different filler contents between 1 and 20 v/v % were prepared and named PPX, where X represents the amount of copper in v/v %. The composites were press molded at 190 °C at 50 bar for 5 min and cooled under pressure by flushing the press with cold water, resulting in a film of about 1 mm thickness. For the different analyses, the samples were cut to obtain dimensions of 20 × 20 mm.

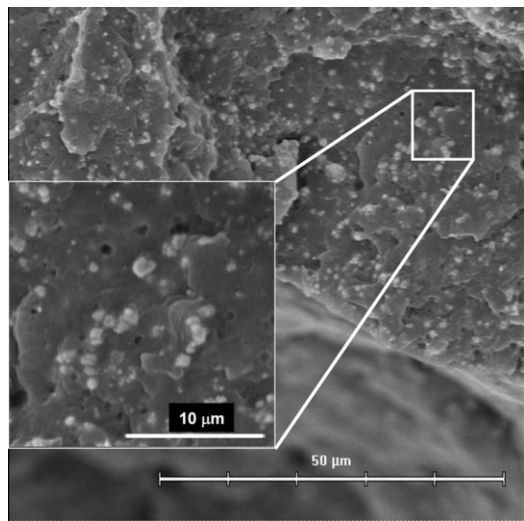
### Composite Characterization

The bacteria were grown overnight in Luria-Broth at 37 °C for the antimicrobial tests. Afterward, the bacterial count was standardized to  $\approx 10^6$  CFU · mL<sup>-1</sup> by measuring their optical density and maintained 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 mL of the bacteria broth was poured into the sample surface and covered with a glass for a specific time ranging from 30 to 360 min at 37 °C. After the time elapsed, the samples were thoroughly washed with a solution of 0.88 wt.-% of NaCl and 1 wt.-% of Tween 80 to remove the surviving bacteria from the surface. From this solution, an aliquot of 40  $\mu$ L was extracted, transferred onto a nutrient agar and incubated for 16 h at 37 °C before colonies were counted. All the experiments were repeated at least twice on different days. The samples, tools, glass materials and wells were sterilized using an Orthmann autoclave for 20 min at 1 bar and 140 °C before any experiments.

The release of Cu<sup>2+</sup> from the nanocomposites was quantified using a Perkin Elmer EAA equipment model 2280. Samples of 10 × 10 mm were immersed in a solution of 100 mL of distilled water and 10 mL from this solution were analyzed at different times. The surface characterization of the composites was carried out by X-ray photoelectron spectroscopy (XPS) in a Physical Electronics 1257 system assisted with argon ion erosion at 4 KeV. Finally, the morphology of the nanocomposites was analyzed using a Tesla BS343A scanning electron microscope (SEM).

## Results and Discussion

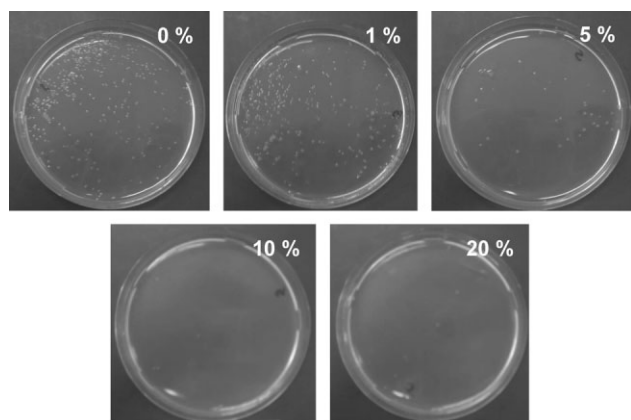
Figure 1 displays a representative SEM image of the composites. Despite the 5 nm average size of the original



**Figure 1.** A representative SEM image of the sample PP5. The copper particles appear as aggregates of the original 5 nm particles.

CNP, they agglomerate, forming secondary structures with sizes ranging from 100 nm to 900 nm that are well dispersed through the polymeric matrix. Primary CNP, if present, cannot be observed due to the resolution of the SEM equipment. Other nanocomposite samples showed a similar morphology.

The presence of bacterial cells was tested every 30 or 60 min up to a total elapsed time of 360 min. Some representative results are displayed in Figure 2 for bacterial counts recovered after 90 min contacting the samples. The presence of CNP drastically reduces the survival of bacteria, compared with the polymer without CNP. The composite with 10% v/v was able to reduce the number of bacteria by more than 99% and 99.9% after only 120 and 180 min of



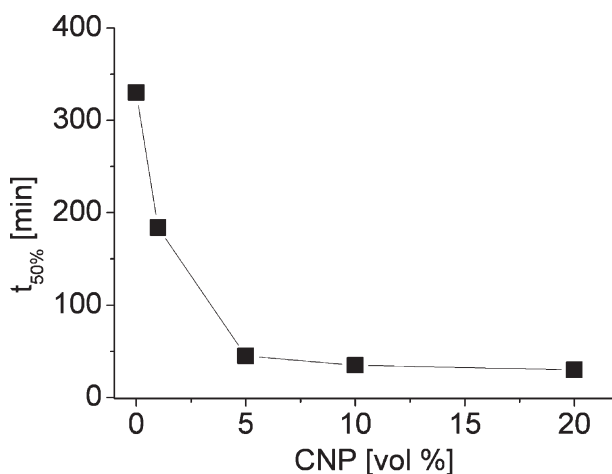
**Figure 2.** Pictures from an optical microscope of the Petri plates showing the survival bacteria for the different composites after 90 min of surface exposition. The number in the top right corner represent the content of CNP [v/v %] of each composite.

contact, respectively. Even the sample with only 1 v/v % of CNP was able to kill 99.9% of the living population after 4 h of contact. Therefore, increasing the amount of nanofiller not only drastically limits the survival of bacteria but also affects the kinetics of the process.

These results show that the addition of CNP to a poly(propylene) matrix results in strong and fast antimicrobial activity to the composites in a controlled way. The antimicrobial results for some of our poly(propylene)/CNP composites are similar to those reported for either pure copper or its alloys under similar microbial tests where the bacteria were killed by surface contact in less than 90 min.<sup>[2,27]</sup>

To improve our quantitative analysis, we have defined the mean time  $t_{50\%}$  as the time needed to kill the 50% of bacteria in contact with the surface of the nanocomposites. Figure 3 depicts the dependence of  $t_{50\%}$  on the concentration of CNP. The PP without CNP needs around 350 min to reduce the bacteria to 50%, representing the normal growth behavior due to nutrient reduction and accumulation of catabolic metabolites. Nevertheless, with only 1 v/v % of CNP this time is reduced by half. Furthermore, in the other composites, the  $t_{50\%}$  is reduced by one order of magnitude showing the strong effect of the CNP on the biocide properties of the composite. Our quantification of the effect of the nanofiller content on the antimicrobial behavior of polymer nanocomposites, summarized in Figure 3, could be of great help for future applications of these materials by rationalizing the use of the nanofiller. In particular, these data will allow the production of tailor-made biocide materials by changing the amount of nanofiller in the polymeric matrix according to specific antimicrobial purposes.

An interesting consequence of the results from Figure 3 is related to the existence of a critical concentration of CNP, which drastically improves the antimicrobial properties of



**Figure 3.** Effect of the CNP content in the polymeric matrix on the time needed to kill 50% of the original bacteria ( $t_{50\%}$ ).

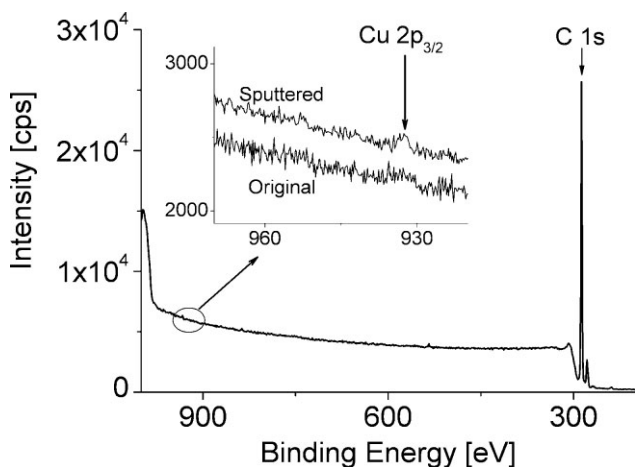
the composite. From our data, samples with concentrations higher than 5 v/v % (30 w/w %) present a stronger and faster antimicrobial properties than samples with lower filler amounts. A similar threshold was reported by Cai et al. for the  $\text{Cu}^{2+}$  release rate in polyethylene/CNP composites, since at a higher copper concentration, a drastic increase of this property was observed.<sup>[23]</sup> This result was associated with the formation of a continuous network of copper nanoparticles in the samples.<sup>[23]</sup> The results from Torsi et al. can also be related with a critical concentration around 10 w/w% for both the copper ion release and the antimicrobial behavior, although using a different type of polymer.<sup>[18]</sup> Therefore, in the same way as for other nanocomposite properties, such as the electrical conductivity or the viscosity, a concentration threshold exists beyond which the antimicrobial behavior dramatically increases. This threshold is lower than that reported for the electrical conductivity in similar systems (around 10 v/v %) as a continuous of copper particles is not required for bactericide effects.<sup>[28,29]</sup> A possible mechanism to understand this threshold is given below.

Normally it is thought that only copper particles near the composite surface can contribute to the biocide properties of the sample. The last hypothesis is consistent with studies where the biocide agents are in contact only with the surface of the polymeric matrix.<sup>[25]</sup> Under this hypothesis, the amount of copper near the surface, either as ions or as nanoparticles should be relevant in our samples as they are able to kill the pathogens. The surface amount of CNP in our nanocomposites was measured with X-ray photoelectron spectroscopy (XPS), a tool sensitive to surface composition. The results for sample PP10 are shown in Figure 4 where the strongest photoelectron line from copper, the  $2p_{3/2}$ , cannot be resolved from the noise. This means that, contrary to what would be expected from the relatively high amount of

copper, the sample does not present relevant evidence of CNP within the surface detection limit of XPS, estimated around 0.1%. After sputtering the surface with argon ions at 4 keV, the signal associated with copper did not change significantly. The same was observed in the other composites. Therefore, the XPS results show that the surface is polymer rich, ruling out the necessity of large amounts of CNS at the surface of the composite to have antimicrobial properties.

Despite the tiny amount of copper at the surface of the samples, the composites are able to produce dissolved  $\text{Cu}^{2+}$  when they are left in water as was quantified in this work. After 6 h of sample immersion, the concentration of  $\text{Cu}^{2+}$  was around  $5 \mu\text{g} \cdot \text{mL}^{-1}$ , increasing with time. Notice that our equipment was able to measure relevant amounts of copper in solution only after 6 h, much longer than the time needed to kill 99.9% of the bacteria. It can be speculated that the specific physico-chemical conditions in the surface of the bacteria and in the media, far away from those of pure water, can accelerate the corrosion process of the CNP, as reported for simulated intrauterine solutions.<sup>[24]</sup> As a matter of fact, it was recently reported that the release of  $\text{Cu}^{2+}$  is facilitated in a nutrient media, probably due to reactions with chloride ions.<sup>[30]</sup>

A plausible mechanism explaining the antimicrobial behavior of the nanocomposites is the corrosion process of CNP from the bulk or the sub-surface region due to the diffusion of water molecules interacting with the surface of these particles. The work of Radheshkumar et al. showed that this mechanism is able to explain the antimicrobial properties of poly(propylene)/silver nanoparticle composites.<sup>[10]</sup> In particular, they showed that the amorphous region of the polymer is very relevant in order to understand these processes. Although poly(propylene) is highly non-polar, molecules of water have been reported to diffuse through it.<sup>[31]</sup> This diffusion is only through interconnected amorphous parts in the poly(propylene) defining a "percolation network".<sup>[31]</sup> This network in the pure material is formed by the different interfaces inside and between the spherulites. As the presence of CNP changes the morphology of the spherulites, in particular by increasing their nucleation density,<sup>[10,32]</sup> the diffusion of water will be higher because of the new formed polymeric interfaces. The CNP/polymer interface can further increase the water diffusion through the holes or micron-scale defects allowing fast Knudsen diffusion.<sup>[33]</sup> It has been reported that one effect of the nanoparticles is disrupting the packing of the polymer chains, thus increasing the accessible free volume in the matrix and therefore facilitating the molecular diffusion.<sup>[33]</sup> These authors postulated that highly networked nanoparticles promote the Knudsen diffusion through the composite, supporting our concept of a threshold concentration for ion release and antimicrobial behavior by means of the water diffusion



**Figure 4.** XPS analysis of the composite PP10 showing that the presence of surface copper is not relevant, even after sputtering process.



processes. Therefore, the formation of a continuous interface polymer/CNP network in the polymer facilitates the diffusion processes explaining our results.

## Conclusion

Poly(propylene)/CNP nanocomposites with filler content between 1 and 20 v/v % were prepared by the melt mixed method, allowing a quantitative measurement of the antimicrobial behavior of CNP. The kinetics of the biocide processes can be controlled by means of the concentration of the filler. Tentatively, it seems possible to establish a threshold CNP concentration which dramatically increases this process. Composites with 1 v/v % are able to kill 99.9% of the bacteria after 4 h of contact. This time is drastically reduced to 2 h in samples with filler contents higher than 10 v/v %. XPS measurements showed no significant concentration of CNP at the surface, therefore the antimicrobial properties must be attributed to bulk or sub-surface copper.

**Acknowledgements:** The authors gratefully acknowledge the financial support of CONICYT through projects FONDECYT INICIACION EN INVESTIGACION 11075001, PSD53 and FONDAP 11980002. The supply of poly(propylene) by Petroquim S.A. and of copper nanoparticles by Versus Productos Industriales S.A. (Chile) are also gratefully acknowledged. We express our thanks to Dr. F. Pizarro and Dr. A. Neira for the Cu<sup>2+</sup> measurements and SEM images, respectively.

Received: November 2, 2009; Revised: November 17, 2009;  
Published online: January 5, 2010; DOI: 10.1002/marc.200900791

**Keywords:** biomaterials; fillers; nanocomposites; nanoparticles

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