



Nanoparticles as antifungal additives for indoor water borne paints

N. Bellotti^{a,b,*}, R. Romagnoli^{a,b}, C. Quintero^c, C. Domínguez-Wong^c, F. Ruiz^c, C. Deyá^{a,b}

^a CONICET, Centro de Investigación y Desarrollo en Tecnología de Pinturas (CIDEPIINT)-CICBA-CONICET, La Plata, Argentina

^b Universidad Nacional de La Plata, La Plata, Argentina

^c Universidad Autónoma de San Luis Potosí, San Luis Potosí, Mexico

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ABSTRACT

There are nanotechnology-based materials that can be used as antimicrobial additives in different applications such as water-borne paints. Antimicrobial paints are important tool in order to avoid indoor biological colonization and therefore prevent paint bio-deterioration and health problems in people and pets. These paints would have application in kitchens, bathrooms and hospitals. The present study evaluated the incorporation of silver (of two different sizes), copper and zinc oxide nanoparticles in indoor waterborne paints and the bio-resistance imparted by them. The antifungal activity of nanoparticles is a less studied topic in relation to the antibacterial activity but is no less important from the environmental point of view. Molds that grow in indoor environments contribute significantly with bioaerosol formation and therefore on air contamination and human health deterioration. In this sense, this research evaluated the nanoparticles' antifungal activity using previously isolated fungi, *Chaetomium globosum* and *Alternaria alternata*, on solid medium. Then, the bio-resistance of acrylic paints, with nanoparticles incorporated, was evaluated in Petri dishes and observations were made using scanning electron microscopy. The better results were obtained with the paint that contained silver with the smaller size (10 nm).

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1. Introduction

Mold can grow on wide variety of materials when sufficient moisture is available generating great numbers of spores, cell fragments, allergens, mycotoxins, endotoxins, β -glucans and volatile organic compounds [1–3]. An excess level of any of these agents in the indoor environment is a potential health hazard; therefore persistent microbial growth should be avoided or minimized [4]. In this sense, exposure to microbial contaminants has been associated with respiratory symptoms, allergies, asthma and immunological reactions and associated with the 20% of the documented infected cases with sanitary assistance [5–10]. Furthermore, molds growth affects materials causing their deterioration [5,7,9,11]. Wall waterborne paints, commonly acrylic-based, are a target to microorganisms due to the fact that they contain cellulosic compounds as thickeners. These compounds can be used by the microorganisms as carbon source producing degradation of the

paint: stains, color changes, chalking and adhesion loosening can be seen in an attacked paint.

Antimicrobial paints have the main objective to avoid or inhibit microbiological colonization and further growth often due to the incorporation of active agents named biocides [9,12]. The new tendencies in biocides formulation are directed to replace the traditional organochlorides and organometallic compounds by others with less environmental impact [9,13,14]. Antimicrobial coatings help to control infections propagation in health care centers and public places [7] and, despite they do not eliminate such infections transmittal they have the capacity to reduce it significantly [15–17].

Lots of compounds based on nanotechnology can be used as antimicrobial agents for different plastic matrixes, textiles, cosmetics, ceramics, paints, etc. [18]. Selection of appropriate materials is an important key to formulate these kind of coatings, taking this into account, nanomaterials as bioactive additives are promising [19,20]. The wide nanoparticles bioapplication is due to their excellent antibacterial activity on several gram positive and negative bacteria [21]. Silver nanoparticles (NP) exert more efficient than ions and silver salts in mediating their antimicrobial activity [22,23]. Usually, Ag^+ ions are efficient bactericides in low concentrations as low as about 0.001–0.05 $\mu\text{g/mL}$, much lower than the doses for silver toxicity to human cells [24]. Furthermore, published data that examined antibacterial effect of copper and zinc

* Corresponding author at: CONICET, Centro de Investigación y Desarrollo en Tecnología de Pinturas (CIDEPIINT)-CICBA-CONICET, La Plata, Argentina.
Tel.: +54 2214831142.

E-mail address: pinturashigienicas@cidepint.gov.ar (N. Bellotti).

oxide against bacterial biofilm revealed, in certain cases, significant reduction of bacterial growth [24–26].

In the present work, the biocide action of silver (AgA and AgG), copper (Cu) and zinc oxide (ZnO) NP incorporated in interior water-borne paints was evaluated; the size of silver nanoparticles was 10 nm (AgA) and 62 nm (AgG), of while the size of copper and zinc oxide were 20 and 40–50 nm, respectively. Moreover, the Ag nanoparticles were obtained by environmental friendly route using gallic acid as a reducing agent [27]. Gallic acid is a renewable source as it is widespread natural product often characterized by substantial accumulations in certain tissues like young leaves of tea plant (*Camellia sinensis*) [28].

The antifungal activity of the NP was evaluated in solid media employing *Alternaria alternata* and *Chaetomium globosum* as aggressive fungi. Besides, bio-resistances assays were done on agarized media by a procedure based on ASTM D 5590 standard [29].

The molds were selected for their ability to grow on paint films and negative impact on human health [30,31]. In this sense, *Alternaria* and *Chaetomium* are able to produce a variety of different compounds including mycotoxins, which are toxic to mammals, and cellulolytic compounds that deteriorate the water-based paints as they degrade the cellulosic thickeners employed in paint formulation [30,31].

In order to increase the efficiency of the paint, the concentration of the NP was increased obtaining better results, especially in the case of AgA. Scanning electron microscopy (SEM), low vacuum mode, was employed to observe the paint surface exposed to the fungi in the case of the more efficient NP.

Bright and color change of the paints after the addition of the NP were evaluated too.

2. Methodology

2.1. Nanoparticles preparation

AgA: An aqueous solution was prepared dissolving AgNO₃, 0.001 M in 100 mL of deionized water. Later on, 10 mL of deionized water containing 0.01 g of gallic acid were added and the solution was placed under magnetic stirring; the pH value was increased up to 11 with the addition of NH₄OH [32].

AgG: The synthesis was similar to the described before for AgA, but after the addition of gallic acid, the solution was irradiated with UV light (254 nm, 15 W) for 30 min. After that, the solution was heated 30 min at 80 °C [32].

Cu: Copper nanoparticles were synthesized with a precipitation method by using an aqueous solution of CuSO₄ and ethylenediamine as precursors, then, a saturated solution of NaBH₄ was added until copper precipitated with a black color. The nanoparticles obtained were washed with ethyl alcohol [33].

ZnO: A 0.05 M solution of ZnCl₂ was prepared and 2 mL of HCl, 0.1 M were added. The solution was heated up to 75 °C with the addition of 5 g of dextrin as stabilizer. The solution acquired a black, opaque color. When the temperature of the solution decreased to 25 °C, an aqueous solution of NaOH (1 M) was added.

2.2. Nanoparticles characterization

The nanoparticles were characterized with UV–vis spectroscopy using an USB4000 UV–Vis spectrometer from Ocean Optics Inc. and dynamic light scattering (DLS) technique to confirm particle size distribution. Size distribution measurements were made in triplicate with a Malvern Zetasizer Nano ZS Instruments operating with a He–Ne laser at a wavelength of 633 nm and a detection angle of 90°; all samples were analyzed for 60 s at 25 °C. To confirm shape,

each sample was diluted with deionized water and 50 µL of each suspension was placed on a formvar-coated copper grid for transmission electron microscopy (TEM). All samples were analyzed by TEM with a JEOL JEM-1230 at an accelerating voltage of 100 kV and X-ray diffraction (XRD) using GBC MMA SPELLMAN.

2.3. Fungi isolation and identification

Two fungi were collected by swabbing from paint films exposed to indoors environment in La Plata city (34°54' S and 57°55' W) [34]. The culture media (DG-18) for the fungal growth were: 0.5 g protease peptone, 1.0 g glucose, 0.1 g KH₂PO₄, 0.05 g MgSO₄·7H₂O, 1.5 g agar and distilled water up to 100 mL. The DG-18 to fungal isolate additionally contained: rose bengal (2.5 mg/100 mL) to restrict colony spreading without affecting spore germination and streptomycin (30 mg/100 mL) to inhibit bacterial growth. The samples were dispersed in physiological saline solution, dilution series prepared and plating in DG-18 to fungal isolate agar plates and were then incubated at 25 °C. Two of the most frequent fungi observed were selected considering their characteristics according to bibliographic data and kept at 4 °C in DG-18 agar slants. These fungi were identified based on their micro and macro-morphological characteristics, using standard taxonomic keys [35]. Optic microscopy was employed in order to identify the fungi by the observation of their characteristics structures [36]. The fungi were recovered and growth in DG-18 at 25 °C for 20–25 days before used.

2.4. Antifungal activity

Fungal spores were removed from the DG-18 agar and suspended in 0.85% p/v NaCl and 0.005% p/v Tween 20 solution; the concentration of the suspension was adjusted employing a Neubauer chamber to 10⁶ spores/mL [29].

Petri dishes containing the selected NP and DG-18 agar were inoculated with 20 µL of the spores' suspension. The concentration of the NP was 1 × 10^{−4} M. Besides, dishes without NP were prepared as control.

Fungal growth at 25 °C was evaluated every week during 1 month as the average diameter of the colony [37,38]. The relative growth inhibition was calculated by percentage, using the following formula [39]:

$$\text{Inhibition (\%)} = \left(1 - \frac{\text{Radial growth with NP}}{\text{Radial growth of control}} \right) \times 100$$

2.5. Paint bio-resistance assay

The NP were added to a commercial acrylic water-based wall and ceiling paint (Borgolotex®, 25.2% of resin, by weight) in a high speed disperser. The concentration studied at the beginning was 5.8 mg/100 g of paint. Then, given the good results, in the case of AgA, AgG and Cu NP, it was decided to test lower concentrations (0.64 and 1.90 mg/100 g). In the case of ZnO, the results were not so good so it was decided to test a higher concentration (9.6 mg/100 g).

Glass slides (7.5 cm × 2.5 cm) were wiped with ethanol, dried under laboratory conditions (65% relative humidity and 25 °C of temperature) and painted with the tested paints, by brush, on one side. Two layers of paint were applied. The painted slides were kept under laboratory condition for 15 days before testing. Also a series was painted with the acrylic paint without NP, as controls.

Before inoculating with the selected fungi, the painted glasses were cut in squared pieces (2.5 cm × 2.5 cm) and irradiated with a germicide UV Phillips lamp (20 W) for 40 min each side to superficial decontamination [14]. Samples thus obtained were placed in plates containing minimum mineral media before solidification with the painted side up. Therefore the level of the culture medium

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