EUROPEAN ACADEMIC RESEARCH
Vol. IX, Issue 9/ December 2021

Impact Factor: 3.4546 (UIF)
DRJI Value: 5.9 (B+)

ISSN 2286-4822
www.euacademic.org

Evaluation of Antimicrobial Action of Ink Incorporated with Silver Nanoparticles

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Abstract

This study aimed to evaluate the antimicrobial action of a water-based acrylic paint incorporated with silver nanoparticles (AgNPs). AgNPs were synthesized by chemical reduction of Ag^{+} ins from silver nitrate, sodium

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borohydride, as a reducing agent and sodium carboxymethylcellulose stabilizer (CMC) at concentrations 0.3% and 1.0%. The characterization occurred by UV-Vis spectrophotometry, dynamic light scattering technique (DlS), zeta size potential and field emission scanning electron microscopy (SEM-FEG). The antimicrobial action of the nanoparticles were evaluated by diffusion in pit agar against Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Streptococcus pneumoniae and Candida albicans. The ink was incorporated with AgNPs in the 10:03 (v/v) ratio of ink by the suspension of nanoparticles. Ink without adding AgNPs was used as control. Antimicrobial evaluations of nanoparticles and additive inks were performed, respectively, by the method of diffusion in pit and disc agar against the microorganisms Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Streptococcus pneumoniae and Candida albicans. Nanoparticles with and without CMC 0.3% and 1.0% presented sizes around 100 nm as well as polydispersed solutions and high level of colloidal stability. The spherical shape of the nanoparticles is confirmed for synthesis without additional stabilizer and with CMC 1.0%, showing that the pacifying agent did not alter the nanoparticle shape. The antimicrobial activity of dispersions with silver nanoparticles at low concentrations was proven by well diffusion test against the bacteria Klebsiella pneumoniae, Staphylococcus aureus, Streptococcus pneumoniae and the fungus Candida albicans. The disc-diffusion test of the inks showed inhibition only for C. albicans in higher concentration.

Key Words: Antimicrobial ink. Water-based paint. Silver nanoparticles.

INTRODUCTION

The paints used in the internal and external environments of buildings in general, called architectural or real estate paints, are composed of resin, pigments, solvents and additives that, when applied to the substrate, form an opaque and adherent film with the objective of decorating the spaces and protecting the surfaces (FAZENDA, 2009). Real estate paints have great prominence in the paint sector in Brazil, representing 82.7% of the total volume produced in 2018 (ABRAFATI, 2020). At the present time, there is a demand for architectural paints with advanced antimicrobial properties due to new public health challenges.

The inadequate use of antibiotics and chemotherapy has resulted in the increase of microorganisms resistant to these drugs, making patients in health units, especially those in the Intensive Care Unit (ICU), more vulnerable to acquiring nosocomal infections and with higher mortality rates (ANDRADE; LEOPOLDO; HAAS, 2006; OLIVEIRA et al., 2010). A study of 638 patients treated in the ICU for a period of 12 months showed that 68 cases (12.98%) were identified with infection by multidrug-resistant bacteria and had a mortality rate of 50% (ANDRADE; LEOPOLDO; HAAS, 2006). In another group with 1,235 patients also treated in Brazilian ICUs, 761 (61.6%) had infection on the day of the study and a mortality rate of 37.6%, in addition, infected individuals had a significantly longer length of stay in the ICU and hospital than non-infected individuals (SILVA et al., 2012).

Resistant organisms present in hospital units are transmitted from person to person through the hands of health professionals, equipment used by the patient and contaminated surfaces in the health care environment, therefore, adequate disinfection of hospital surfaces is a way to prevent the spread of diseases (STRICH; PALMORE, 2017). These microorganisms have particularities that drive their contamination, such as viability for long periods of time, including on dry surfaces that make the transmission cycle long-lasting (STRICH; PALMORE, 2017).

Another circumstance of risk to people's health is the formation of fungi on the surfaces of internal environments, which are prolonged by users in many situations. Fungi cause allergic, respiratory and infectious diseases through contact or inhalation of spores and mycotoxins, and are found adhered to the surface of materials or aerosolized and polluting the internal air (LI; YANG, 2004).

The advancement of nanotechnology and its application to improve the qualities of materials in general have made silver nanoparticles one of the most researched materials with a wide field of employability. These nanoparticles have interesting properties regarding chemical stability, malleability, flexibility, high electrical and thermal conductivity, catalytic activity, relatively low production

cost and remarkable antimicrobial action against bacteria, viruses, fungi and protozoa (DURÁN, 2019).

The antibacterial action of silver nanoparticles can result in cellular inactivation, caused by the interaction of silver with the sulfur of proteins and amino acids, and inhibition of enzymatic activities, due to the interaction of silver ion released by nanoparticles with DNA phosphorus (DESHMUKH, 2018). Silver nanoparticles can also damage the bacterial membrane, through accumulation on its surface followed by penetration into bacteria, forming fossa, altering permeability and leaking bacterial content (ZHENG et al., 2018). In addition, severe cellular damage can be generated by oxygen-derived free radicals, Reactive Oxygen Species (ROS) (LEMIRE; HARISSON; TURNER, 2013).

Given the importance of the search for new biocides as additives in real estate paints to control disease transmission, this study aimed to evaluate the antimicrobial action of acrylic paint incorporated with silver nanoparticles.

MATERIALS AND METHODS

Materials

The synthesis of the nanoparticles employed the reagents silver nitrate ($AgNO_3$) (Sigma-Aldrich), sodium borohydride ($NaBH_4$) (Aldrich) and the stabilizer carboxymethylcellulose sodium (CMC) (Zibo Hailan). Before the experiments, the entire glasshouse was cleaned with alcoholic potash and distilled water.

The ink used in the research is a Brazilian commercial product of standard acrylic type and brown matte color (chocolate), composed of resin based on aqueous dispersion of acrylic styrene copolymer, glycols, ethoxylated and carboxylated tensors, pigments free of heavy metals, inert loads and water. The ink has a specific weight of 1,350 to 1,450 g/cm^3, volatile organic compounds (with the exception of water) at 15.5 g/L, viscosity between 95-105 UK and solids concentration by weight of 55-60%. Ultra purified water by the Gehaka Master System 2000 equipment was used as a solvent in ink synthesis and preparation solutions.

Synthesis of AgNPs

The process of synthesis of silver nanoparticles was performed by chemical reduction, according to Junior et al. (2012) and Solomon et al. (2007), in which silver nitrate ($AgNO_3$) acts as the precursor material and sodium borohydride ($NaBH_4$) as the reducing agent.

Initially, a volume of 75 mL of solution with 0.002M concentration and 12.5 mL of 1.0% sodic carboxymethylcellulose (CMC) solution was added to a 250 mL Erlenmeyer. The container was placed in an ice bath for 15 minutes. Under vigorous magnetic agitation, silver was reduced with a drip of 25 mL of 0.001M solution in Erlenmeyer with and CMC. The synthesis occurred in triplicate to ensure its reproducibility.

Characterization of AgNPs

The confirmation of the obtaining of the nanoparticles and the estimation of their sizes was made by the ultraviolet-visible spectra (UV-Vis) measured in the region of 300 to 600 nm by a UV-Vis spectrophotometer, Uv-1650PC model of Shimadzu.

The determination of the hydrodynamic diameter (Z-average), size distribution and polydispersivity index (PDI) of the nanoparticles were performed by the dynamic light scattering technique (DLS) with malvern's Zetasizer nano-ZS90, an equipment that also measured zeta potential for stability verification. These parameters measured by Zetasizer were performed in triplicate for each of the three prepared solutions.

For the analysis of the shape of the nanoparticles, images were generated by a JEOL JSM-7100F field emission scanning electron microscope (SV-FEG) with a Deben Gen5 transmitted electron detector (STEM) with electron acceleration voltage of 30 kV. The samples were deposited on 400 mesh copper mesh covered with carbon film.

Antimicrobial test of AgNPs

The antimicrobial activity of the nanoparticles was determined by the well agar diffusion method, adapted from NCCLS (2003), against *bacteria Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC

4352), *Staphylococcus aureus* (ATCC 25923), *Streptococcus pneumoniae* (ATCC 49619) and against the fungus *Candida albicans* (ATCC 24433). The bacteria and *C. albicans* were cultured at 37 ºC in luria-bertani medium for 4 to 6 h and had turbidity adjusted on the Mc Farland scale at 0.5 for the bacteria and 1.0 for the fungus. Then, Petri dishes with Mueller Hinton or Sabouraud Dextrose agar were inoculated by a sterile swab with bacteria and C. *albicans,* respectively. With the aid of a mold, wells were made in petri dishes and filled with 20 µL and50 µL of silver nanoparticle suspensions. Then, these plates were incubated for 24 h in the greenhouse at 37 ºC. The solutions that showed inhibition around the well, had their halos measured in millimeters with the aid of a millimeter ruler. The tests were performed in triplicate.

Incorporation of nanoparticles in ink

The addition of silver nanoparticles in water-based acrylic paint occurred at different concentrations so that it was possible to evaluate the effect of nanoparticles on the antimicrobial action of tinta. The determination of concentrations was defined in proportions of volume measurements, according to table 01. Given the synthesis route of the nanoparticles and the proportion of addition of the AgNPs solution, the amount of silver at concentration 02 is 5.53 µg/mL. Concentration 01 was defined as a blank sample, without the addition of nanoparticles.

Table 01 - Concentrations a tested.

Sample	Composition	Volume measurements
Concentration 01	Acrylic paint	10
	Water	03
Concentration 02	Acrylic paint	10
	Silver NPs Solution	03

Ink antimicrobial test

The antimicrobial activity of the ink was determined by the disk agar diffusion method, according to NCCLS (2003), against *bacteria Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 4352), *Staphylococcus aureus* (ATCC 25923), *Streptococcus pneumoniae*

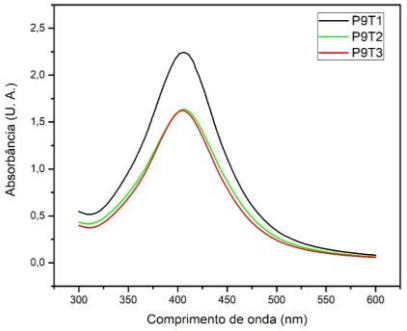
(ATCC 49619) and against the fungus *Candida albicans* (ATCC 24433). The bacteria and *C. albicans* were cultured at 37 ºC in luria-bertani medium for 4 to 6 h and had turbidity adjusted on the Mc Farland scale at 0.5 for the bacteria and 1.0 for the fungus. Then, Petri dishes with Mueller Hinton or Sabouraud Dextrose agar were inoculated by a sterile swab with bacteria and C. *albicans,* respectively. Paper discs of 6 mm in diameter received an aliquot of 50 µL of the ink at the tested concentration and, soon after, they were placed on the surface of the culture plates. These plates were incubated for 24 h in the greenhouse at 37 ºC. The solutions that showed inhibition around the discs had their halos measured in millimeters with the aid of a millimeter ruler. All tests were performed in quintuplicate.

RESULTS AND DISCUSSION

Characterization of Silver Nanoparticles

The UV-Vis spectra resulting from synthesized suspensions present maximum absorbance peaks at 405.7 ± 0.5 nm, according to Figure 01. These values are characteristic of nanoparticles and confirm their formation. In addition, it is noticeable that the peaks have a width at half height higher, which is due to the high polydispersivity of the nanoparticles(AGNIHOTRI; MUKHERJI; MUKHERJI, 2014).

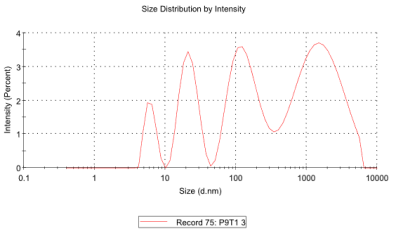
Figura 01 – Espectros de UV-Vis das triplacatas de nanopartículas de prata.



The suspensions showed silver nanoparticles with a hydrodynamic diameter equal to 113.2 ± 22.1 nm, but a considerable portion of this size may result from the CMC stabilizer layer. In studies in which the measurement of the size of polymer-stabilized nanoparticles was performed by Transmission Electron Microscopy (MET) and DLS, there was a considerable difference in the diameters measured in each, since met is capable of measuring only the metallic nucleus, while DLS includes polymeric coating (ARAGÃO, et al., 2016; HILEUSKAYA, et al., 2020).

The size distribution of the nanoparticles is irregular and polydisperse, as shown in Figure 02. The polydispersivity index varies between 0 and 1, with values close to 1 for polydispersed samples and close to 0 for monodisperses (KAUR; GOYAL; KUMAR, 2018). Reaffirming the UV-Vis analysis, the nanoparticles exhibited PDI equal to 0.933 ± 0.073.

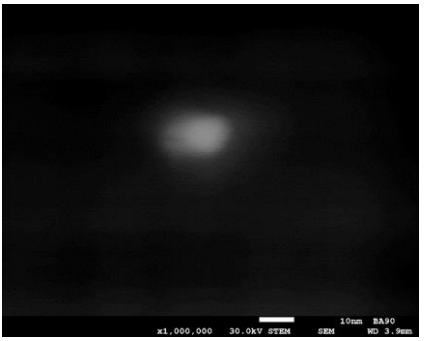
Figura 02 – Distribuição de tamanho medidas por DLS. b



The samples produced a zeta negative potential of -59.1 ± 5.7 mV, suggesting an electrostatic stabilization by effect of the CMC stabilizer that coated the nanoparticles. According to TUAN et al. (2015), zeta potential values greater than +30 mV or less than -30 mV represent a high colloidal stability, so nanoparticles stabilized with CMC are considerably stable.

The Image of Mev-FEG of the AgNPs, figure 03, shows its spherical shape, expected result for synthesis by chemical reduction of Ag^{+} from $AgNO_3$ e $NaBH_4$(JUNIOR et al., 2012; SOLOMON et al., 2007). Therefore, the stabilizer did not change the shape of the nanoparticles.

Figure 03 - MEV-FEG image of nanoparticles.



Evaluation of antimicrobial activity of Silver Nanoparticles

The verification of antimicrobial activity by well agar diffusion test resulted in the inhibition halos in table 02. It is noticed that by changing the volume between 20 µL and 50 µL of the solution of the same concentration of AgNPs, there is a different response for microorganisms, except for *E. coli* that did not present inhibition in any sample. The fungicide action of AgNPs was tested only with 20 µL of solution, which presented a halo of 11.7 ± 0.5 mm.

Table 02 - Values of the diameter of the inhibition zone of the AgNPs on average a standard deviation (mm). wi = without inhibition.

Microorganism	20 µL of solution	50 µL of solution
Escherichia coli	wi	wi
Klebsiella pneumoniae	wi	9,3 ± 0,9
Staphylococcus aureus	14,0 ± 0,0	5,3 ± 3,8
Streptococcus pneumoniae	6,7 ± 4,7	12,7 ± 3,8
Candida albicans	11,7 ± 0,5	-

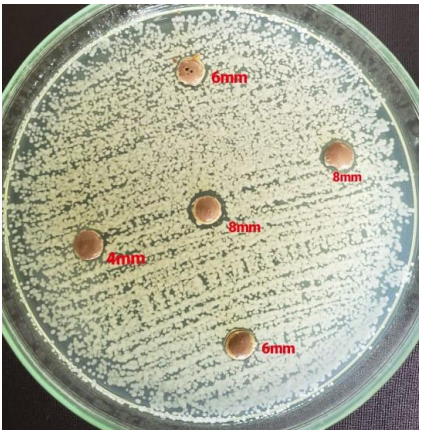
Evaluation of antimicrobial activity of ink with AgNPs

The results of the ink diffusion disc test are in table 03. Commercial water-based acrylic paint without the addition of nanoparticles (concentration 01) showed no injunction against microorganisms. Concentration 02 showed inhibition for *Candida albicans* (figure 04).

Table 03 - Values b standard deviation (mm). wi = without inhibition.

Sample	*Escherichia coli*	*Klebsiella pneumoniae*	*Staphylococcus aureus*	*Streptococcus pneumoniae*	*Candida albicans*
Concentration 01	wi	wi	wi	wi	wi
Concentration 02	wi	wi	wi	wi	6,4 ± 1,5

Figure 04 - Diffusion disk test for concentration 02 against *C. acbicans.*



In the study by Holtz et al. (2012) when analyzing the antibacterial activity of commercial water-based ink added in 1% (m/v) of nanostructured silver vanadate, it was clearly verified the formation of an inhibition halo around the glass plate painted contra *Staphylococcus aureus* Methicillin-Resistant. It is important to highlight that the silver concentration used in the formulation of paints was low, being 5.53 µg/mL for the concentration 02. The addition by Holtz et al. (2012) additive (1g/100mL) represents a concentration approximately 2,000 times higher than that tested in this work.

The growth of microorganisms *Listeria monocytogenes, Salmonella senftenberg, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus subtilis* and *Aspergillus niger* were significantly inhibited in additive PVA latex ink, with silver NPs partially coating zinc oxide NPs at concentrations 0.15% and 0.30%(TORNERO et al., 2018). Fiori et al. (2017) also obtained excellent antimicrobial activity

against Staphylococcus aureus and *Escherichia coli* in acrylic paints additive in 0.4%, 0.8% and 1.2% zinc oxide nanoparticles.

The fungal bioresistance of water-based acrylic paint with silver nanoparticles showed higher efficiency at higher concentrations of nanoparticles, in addition, there was no inhibition halo around the painted glass, indicating the absence of leaching of the material (BELLOTTI et al., 2015). Thus, new ink formulations with higher concentrations of synthesized silver nanoparticles should be performed, since these nanoparticles showed growth inhibition against microorganisms *K. pneumoniae, S. aureus, S. pneumoniae* and *C. albicans*.

CONCLUSION

The nanoparticles with and without CMC 0.3% and 1.0% presented sizes around 100 nm as well as polydispersed solutions and high level of colloidal stability. The spherical shape of the nanoparticles is confirmed for synthesis without additional stabilizer and with CMC 1.0%, showing that the stabilizer did not alter the nanoparticle format. The antimicrobial activity of suspensions with silver nanoparticles coated with CMC at low concentrations was proven by well diffusion test against the bacteria Klebsiella pneumoniae, Staphylococcus aureus, Streptococcus pneumoniae and the fungus Candida albicans. Escherichia coli showed no inhibition in the tested concentration. The two concentrations of formulated inks were tested against these same microorganisms, in which inhibition halo was only formulated for C. albicans at concentration 02.

Acknowledgements
We thank the Coordination for the Improvement of Higher Education Personnel (CAPES) for the master's scholarship.

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