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ANTIBACTERIAL EFFICIENCY OF SILVER AND ZINC-OXIDE NANOPARTICLES IN ACRYLATE COATING FOR SURFACE TREATMENT OF WOODEN COMPOSITES

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ABSTRACT

The paper deals with antibacterial effect of silver and zinc-oxide nanoparticles in acrylic coatings applied at treatment of commercial wooden composites - particleboard and medium density fibreboard. The silver nanoparticles usually better suppressed the activity of the Gram-negative bacterium *Escherichia coli* and the Gram-positive bacterium *Staphylococcus aureus* already at lower concentrations (0.04 – 0.2 mg Ag/100 g of coating) as the zinc-oxide nanoparticles used in 100-times higher concentrations (4 – 20 mg ZnO/100 g of coating). Both types of applied nanoparticles at higher concentrations had a more apparent efficiency against *E. coli* comparing to *S. aureus*.

KEYWORDS: Wooden composites, acryl coating, nanoparticles, silver, zinc-oxide, *Staphylococcus aureus, Escherichia coli*.

INTRODUCTION

The wood-based materials such as plywood, particleboard, fibreboard or WPC need regular maintenance and care of their surfaces to raise persistency against climatic influences, including action of bacteria, molds, staining-fungi or decaying fungi. Their infestation by microorganism and the associated biological degradation are adverse, because performance characteristics such as aesthetic appearance or mechanical properties can be lowered. Another critical disadvantage caused by a microbial colonization of the material surface can be health hazards such as bacterial infection (Gerullis et al. 2018).

Bacteria are naturally found in clinical and industrial settings in association with surfaces. Bacterial surface contamination, i.e. the adhesion, persistence, and colonization of surfaces by bacteria, is increasingly recognized as detrimental to health and society. Improving hygienic standards in many parts of the world allow infectious diseases to be increasingly better controlled. Aside from contagion through contaminated air and direct contact with infected people or animals, contaminated objects play an important role in spreading of infectious diseases. The antibacterial and hygienic properties of material surfaces are therefore important (Kandelbauer and Windsten 2009).

Surfaces of raw commercial wooden composites (CWCs), which usually don't contain biocides or don't release higher amounts of free formaldehyde from amino- or phenol-formaldehyde resins applied at their production, can be inhabited by bacteria and microscopic fungi (Vidholdová et al. 2015).

However, microbial infection can occur as well as on surfaces of laminated and painted CWCs (Nosáľ and Reinprecht 2017, Vidholdová et al. 2015), mainly if they are contaminated with foodstuffs and other organic substances (Nosáľ and Reinprecht 2017). Laminated and painted surfaces of CWCs with antimicrobial effect would reduce the risk of the bacterial and mold infection spreading. Melamine resins are commonly used for lamination or painting of CWCs products for interior applications such as work surfaces, furniture, and laminated wood flooring. Despite their comparatively good cleanability, there is a need for improving their antimicrobial properties (Kandelbauer and Windsten 2009, Kim and Kim 2006, Nosáľ and Reinprecht 2017). Similarly, acryl-based coatings and resins for treatment of wooden and other materials are also susceptible to attacks by microorganisms (Bellotti et al. 2015, Reinprecht and Vidholdová 2017).

Nanomaterials may be strategically advantageous as active antibacterial groups since their surface area is exceedingly large relative to their size, i.e. nanosized particles may provide high biocidal activity although only a small dose of the particles is used (Beyth et al. 2015). Potential of various metal and other nanoparticles as antimicrobial agents was described by more researchers (e.g. Bellotti et al. 2015, Clausen et al. 2009, Holtz et al. 2012, Martínez-Castañón et al. 2008, Moritz and Geszke-Moritz 2013, Nosáľ and Reinprecht 2017, Reinprecht and Vidholdová 2017, Stanković et al. 2013) and others. The metal oxide nanoparticles with antimicrobial activity offer a wide spectrum of applications as additives for biopolymers, medicine, orthodontic, textiles, interior paints and UV-blocking agents. These nanomaterials display an inherent particle-size-dependent antibacterial activity, chemical stability, thermal resistance, toxicity and long-lasting action in comparison to organic antibacterial agents (Negi et al. 2012).

The antibacterial effect of nanoparticles depends on chemical composition, concentration, average particle size, and shape. As the nanoparticle size decreases, they have stronger bactericidal activity (Bellotti et al. 2015, Durán et al. 2016, Guzman et al. 2012, Rai et al. 2009, Rai et al. 2012).

Silver nanoparticles have high efficiency against Gram-positive and Gram-negative bacteria (*Escherichia coli, Staphylococcus aureus, Staphylococcus epidermis, Pseudomonas aeruginosa, Bacillus subtilis*, etc.) (Hsueh et al. 2015, Cho et al. 2005, Kim et al. 2011, Maiti et al. 2014, Maliszewska and Sadowski 2009, Salomoni et al. 2017, Sharma et al. 2009). For example, antimicrobial efficacy of Ag nanoparticles in transparent wood-coatings for outdoor applications tested (Künninger et al. 2014).

Zinc-oxide nanoparticles have also antimicrobial properties (Esparza-González et al. 2016, Kumar et al. 2017, Vidholdová et al. 2015). Interesting is knowledge that ZnO nanoparticles in combination with 3-aminopropyltriethoxysilane are preferred for inducing antimicrobial effects against Gram-positive bacteria without increasing eukaryotic cell toxicity (Esparza-González et al. 2016). Wood/ZnO hybrid materials have a promising future for the antibacterial efficacy on *E. coli*, and zinc-oxide improve also their thermal stability and UV resistance (Dong et al. 2017).

In this work, surface modification of two CWCs – particleboard and medium density fibreboard – was performed with Ag and ZnO nanoparticles added to acrylate coating, with the aim to increase their resistance to bacteria.

MATERIALS AND METHODS

Silver and zinc-oxide nanoparticles

Silver nanoparticles (0.02 mg·mL^{-1} Ag in aqueous buffer containing sodium citrate as a stabilizer; Sigma-Aldrich Co. Ltd., Saint Louis, USA) had the following characteristics: 20 ± 4 nm average diameter determined by transmission electron microscopy.

Zinc oxide nanoparticles (500 mg·mL^{-1} ZnO in aqueous dispersion system; Sigma-Aldrich Co. Ltd., Munich, Germany) had these characteristics: < 35 nm average particle size (APS); < 100 nm length particle size (DLS).

Acrylic coating

One-component acrylic water-soluble clear coating – lacquer Lacroma Clear 50 EM1157-0050 (32 ± 1 wt. % of solid mass in water; Sherwin-Williams, Sweden) had the following application characteristics: density 1030 kg·m^{-3}; viscosity 60-70 s at 23°C by DIN 4.

The acrylic coating was modified with silver nanoparticles in concentrations of: A1) 0.04 mg Ag/100 g of coating; B1) 0.12 mg Ag/100 g of coating; C1) 0.2 mg Ag/100 g of coating, or with zinc-oxide nanoparticles in concentrations of: A2) 4 mg ZnO/100 g of coating; B2) 12 mg ZnO/100 g of coating; C2) 20 mg ZnO/100 g of coating. Application characteristics (density, viscosity) of acrylic coatings did not change due to these modifications.

Wooden composites

Two types of commercial wooden composites (CWC) produced by Kronospan corporation were used as an underlying material for acrylic coatings, i.e. the particleboard No 1 (PB-1) and the medium density fibreboard No 1 (MDF-1), while the PB-2 from the same producer was as the third type of CWC (characterized by a lower content of formaldehyde) used only at bacterial tests as a reference board (Tab. 1).

PBs had thickness 18 mm and MDF 16 mm. The top surfaces of all wooden compo-sites were before painting or direct submitting to bacterial tests sanded gradually with P100, P120 and P150 wide-belt sandpapers.

Painting of wooden composites

Acrylic coatings were applied on the top surfaces of CWC in two spraying layers, each in amount of 100 g ± 10 g per m^2. The first layer was made with the coating Lacroma Clear 50 that was then cured 5 minutes at a temperature of 50°C in air-jet-dryer tunnel and sanded with P320 sandpaper. The second layer was made with one of the modified acrylic coatings containing silver or zinc-oxide nanoparticles then cured 5 minutes at a temperature of 50°C in air-jet-dryer tunnel.

Bacterial activity

The *Staphylococcus aureus* ATCC-25923 as a representative of Gram-positive bacteria and the *Escherichia coli* ATCC-25922 as a representative of Gram-negative bacteria (from collection of microorganisms at the Department of Clinical Microbiology of Hospital Zvolen, Slovakia) were used for bacterial tests.

The top surfaces of CWCs, i.e. raw or painted with acrylic coatings, were cleaned with alcohol solution (8.8:1.2 of ethanol and 2-propanol). Samples of CWCs were then at sterile conditions placed into Petri dishes and following inoculated with 0.1 mL of bacterial suspension with density of 0.5* according to the McFarland scale (1.5×10^8 CFU/mL). Incubation of bacteria on the top surfaces of CWCs was performed at 37°C for 48 h. Bacteria were then stripped from the individual surfaces of raw or painted CWCs using a sterile swap and taken up in a liquid culture medium for 48 h. Finally, the bacteria were pre-inoculated from the liquid medium into the sodium chloride diagnostic soil in the Petri dishes.

The anti-bacterial resistance of the raw and painted (having in acryl coating different amounts of Ag and ZnO nanoparticles) CWCs was assessed by the diagnostic soil method (Nosáľ and Reinprecht 2017, Vidholdová et al. 2015), measuring the bacterial in CFU/mL.

RESULTS AND DISCUSSION

Activity of the bacteria *Staphylococcus aureus* and *Escherichia coli* on the sanded top surfaces of raw commercial wooden composites (CWCs) was influenced by the acceptable content of formaldehyde, having the antibacterial effect as well. PB-1 and MDF-1, which had a higher maximal limit of CH_2O (6.5 mg/100 g of the oven dry CWC by EN ISO 12460-5), were not or only slightly attacked by bacteria whose activity ranged from 0.00 up to 0.07 $\times 10^8$ CFU/mL. On the contrary, PB-2 with a smaller maximal limit of CH_2O (4.0 mg/100 g of the oven dry CWC) was already attacked by both types of bacteria whose activity ranged from 0.10 up to 0.19 $\times 10^8$ CFU/mL.

Results related to the bacterial resistance of the painted CWCs are documented in Tabs. 2 and 3. Bacterial activity of *S. aureus* and *E. coli* on the top surfaces of PB-1 and MDF-1 painted with basic and modified (addition of Ag and ZnO nanoparticles) acrylic coatings ranged from 0.00 up to 0.24 $\times 10^8$ CFU/mL. The basic acrylic coating, i.e. without nanoparticles, did not protect the top surfaces of CWCs against bacterial attacks, but rather it had an opposite effect comparing to bacterial resistance of raw CWCs (see Tab. 1, Tab. 2 and Tab. 3). It was probably due to the absence of antibacterial formaldehyde molecules in the top surfaces of painted CWCs. On the other hand, the modified acrylic coatings, i.e. with addition of Ag nanoparticles (from 0.04 to 0.20 mg/100 g of coating) or ZnO nanoparticles (from 4 to 20 mg/100 g coating) were in more cases better able to withstand bacterial attacks as the pure acrylic coatings.

Tab. 1: Bacterial activity of S. aureus and E. coli on the top surfaces of sanded commercial raw wooden composites (PBs, MDF).

Raw wooden composite	Formaldehyde EN ISO 12460-5 (EN 120) (mg/100 g oven dry board)	*Staphylococcus aureus* (10^8 CFU/mL)	*Escherichia coli* (10^8 CFU/mL)
PB-1	< 6.5	0	0
PB-2	< 4.0	0.19 (0.02)	0.10 (0.02)
MDF-1	< 6.5	0.07 (0.01)	0

Note: Average values are from 4 measurements. Standard deviations are in the parentheses.

The silver nanoparticles had better antibacterial efficiency comparing to the zinc-oxide nanoparticles. It can be seen from a stronger suppression of both bacteria even at application of 100-times lower Ag concentrations (Tab. 2 and Tab. 3). Similar knowledge obtained Bellotti et al. (2015), when activity of microscopic fungi *Chaetomium globosum* and *Alternaria alternate* was more apparently suppressed by Ag nanoparticles comparing to ZnO ones. In their study, Ag nanoparticles with a lower average size of 10 nm were more effective as Ag with average size of 62 nm. There are many mechanisms attributed to bactericidal activity of silver nanoparticles which are still not fully under-stood or cannot be generalized as the nanoparticles are found to act on different organisms differently (Prabhu and Poulose 2012). By Ouay and Stellacci (2015) surface properties of Ag nanoparticles have a crucial impact on their potency, as they influence both physical (aggregation, affinity for bacterial membrane, etc.) and chemical (dissolution, passivation, etc.) phenomena.

Tab. 2: Bacterial activity of S. aureus and E. coli on the top surfaces of commercial wooden composites (PB-1, MDF-1) painted with acrylate coatings containing different amounts of Ag nanoparticles.

Ag nanoparticles (mg/100 g of coating)	PB-1	MDF-1
Staphylococcus aureus (10^8 CFU/mL)		
0	0.18 (0.01)	0.24 (0.02)
0.04	0.16 (0.01)	0.11 (0.04)
0.12	0	0.15 (0.01)
0.20	0	0.14 (0.01)
Escherichia coli (10^8 CFU/mL)		
0	0.20 (0.01)	0.17 (0.01)
0.04	0.16 (0.01)	0
0.12	0	0
0.20	0.03 (0.04)	0

Note: Average values are from 4 measurements. Standard deviations are in the parentheses.

The bacterial resistance of painted CWCs was partly influenced as well as by the type of underlying composite as well (Tab. 2 and Tab. 3). However, we were not able to scientifically explain this knowledge.

Tab. 3: Bacterial activity of S. aureus and E. coli on the top surfaces of commercial wooden composites (PB-1, MDF-1) painted with acrylate coatings containing different amounts of ZnO nanoparticles.

ZnO nanoparticles (mg/100 g of coating)	PB-1	MDF-1
Staphylococcus aureus (10^8 CFU/mL)		
0	0.18 (0.02)	0.24 (0.02)
4	0.23 (0.01)	0.14 (0.02)
12	0.20 (0.01)	0.14 (0.01)
20	0.17 (0.01)	0.07 (0.01)
Escherichia coli (10^8 CFU/mL)		
0	0.20 (0.01)	0.17 (0.02)
4	0.19 (0.01)	0.08 (0.06)
12	0.11 (0.01)	0.13 (0.01)
20	0.05 (0.01)	0

Note: Average values are from 4 measurements. Standard deviations are in the parentheses.

Generally, both Ag and ZnO nanoparticles had a partly better biocidal efficacy against the Gram-negative bacterium *E. coli*. as against the Gram-positive bacterium *S. aureus* (Tab. 2 and Tab. 3).

CONCLUSIONS

Antibacterial resistance of the raw commercial wooden composites (CWCs) – particleboard (PB) and medium density fibreboard (MDF) – was higher if those ones containing a higher acceptable content of formaldehyde.

Antibacterial resistance of the acryl-coated CWCs increased with a higher content of silver and zinc-oxide nanoparticles in their surfaces.

Ag nanoparticles used in acrylic coatings in 100-times lower concentrations (0.04 – 0.20 mg Ag/100 g of coating) had despite this fact a slightly better antibacterial efficiency in comparison with ZnO nanoparticles (4 – 20 mg ZnO/100 g of coating).

Ag and ZnO nanoparticles were partly more effective against the Gram-negative bacterium *Escherichia coli* comparing to the Gram-positive bacterium *Staphylococcus aureus*.

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