

Building Materials with Antifungal Efficacy Enriched with Silver Nanoparticles

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Abstract

Microbiological tests were carried out against fungal spores (*Cladosporium cladosporioides*, *Alternaria alternate*, *Rhodotorula mucilaginosa*, *Aspergillus Niger* and *Geotrichium candidum*). Nanosilver suspension at a concentration of 5-15 ppm was used as a biocidal agent. It was a component of various kinds of tested paint coatings. Studies *Geotrichium candidum* confirmed the high biocidal efficacy thus nanosilver additive for paints and coatings makes it possible to prevent the growth of microorganisms.

Keywords: Bio deterioration; Microbial and mycological resistance; Protection coatings; Paints; Microbiological corrosion

Introduction

Applied modern construction materials, including plaster, primers and especially paints should provide adequate quality through proper maintenance and stability of the product before being installed, and in particular the health safety of the finished product which is used as a coating. The synergistic effect of various external and internal factors promotes biocorrosion, the process of destruction of building materials by microorganisms. The "Encyclopedia of Bioprocess Technology" defines biological corrosion as degradation of materials by the action of living organisms and / or their metabolic activity [1]. Most often biocorrosion is caused by bacteria and microscopic fungi, which leads to the development of biodeterioration or adverse modification and loss of optimal performance [2]. Microbial colonization in painted buildings causes aesthetic problems and may lead to degradation of the coating and splintering [3]. Much more dangerous effects may be caused by the influence of mould on human health. Spores and mycelial fragments escaping from the ground, together with bacteria and dust, form bioaerosols that are a source of air pollution. They may contain toxic compounds called mycotoxins, which cause allergies. *Penicillium spp*, *Aspergillus spp* and *Alternaria spp* are significantly responsible for the formation of many of the allergic symptoms and respiratory diseases in people exposed to high levels of mould spores and allergens.

Penicillium spp is the most frequently isolated fungus from infected internal building environments [4,5]. In research conducted in archives and museums, it was found that these fungi dominated among isolated populations found on the walls and furniture (86.0–99.9%). The most commonly isolated fungi were as follows: *Alternaria spp* (such as *Alternaria alternata*), *Aspergillus spp* (e.g. *Aspergillus versicolor*), *Cladosporium spp* (e.g. *Cladosporium herbarum*), *Penicillium spp* (e.g. *Penicillium carneum*) and *Rhizopus Nigricans*. *B. subtilis*, *Micrococcus species*, *Staphylococcus xylosus*, *Staphylococcus lentus*, *Sphingomonas paucimobilis* and *Pseudomonas alcaligenes* were also isolated, among other bacteria [6].

Uniquely worded European requirements for building construction in terms of hygiene and sanitation as well as biological corrosion resistance were included in the European Communities Council Directive 89/106/EEC (Section 4, &322.2) [7]. They oblige the use of materials, products and building elements that are resistant to fungus

and other forms of degradation. In accordance with basic requirement No. 3 contained in the Interpretative Document on the approximation of the laws and legal acts to that Directive, building structures shall not constitute a health risk to users or the environment [8]. The fulfilment of this criterion by the product is the basis for eco-labelling, which seems particularly valuable to this developing industry in the context of promoting environmentally friendly products.

Depending on the mechanism of effective action, there are three main types of coatings: inhibiting adhesion by and preventing the formation of biofilms, gradually releasing biocides into paints and the environment, and the killing of microorganisms in the case of direct contact [9-12]. From the point of view of the need to provide and maintain an adequate anti-microbial and mycological resistance in dried paint coatings, areas of application are classified as follows: medicine, pharmacy, food and electronics (bio-clean rooms with higher requirements for resistance to antibiotic-resistant strains), facilities for public use under special physicochemical conditions: high humidity, the optimum temperature for growth of microorganisms (bathrooms, production halls, facilities in specific climatic conditions, archives, museums), Facilities for public use under normal physicochemical conditions (residences).

Selection of an appropriate antimicrobial coating for the specific area of application should therefore take into consideration its mechanism of effective action and effectiveness, the scale of the exposure to microorganisms, environmental protection and human health. Currently, in order to ensure adequate protection, active biocidal substances, which are chemicals compounds with a broad spectrum of activity that may also have harmful effects on human health and the environment, are mainly used. The concentration of biocides in paints

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is typically 0.5% wt. Prolongation of the coating protection time can be obtained only by increasing the concentration of biocide. Water-soluble biocides are also prone to excessive leaching, thus high concentrations are used to achieve effective protection of surfaces from biodeterioration [13]. However, this may change the mechanical properties of the coating [14]. There is no universal biocide or active substance that would be compatible with all components of paints and coatings and would also meet the requirements of manufacturers. Some authors [15] suggest that in order to ensure the protection of dried waterborne coatings, twice as much of water-soluble biocides such as 2-n-octyl-4-isothiazolin-3-one (OIT) and 3-iodo-2-propylbutyl carbamate (IPBC) be applied. Isothiazolinone, which is included in a standard patch test panel on contact allergies, is the most widely used biocide for paint protection. Carbendazim is a crucial biocide used to protect dry paint film, but recently introduced legal changes restrict the application of this and other products [16]. Moreover, carbendazim does not inhibit a commonly occurring fungus, *Alternaria* [15]. Mixtures of biocides that include combinations of fungicides and algacides are often used to correct deficiencies in the fungicidal spectrum, e.g. IPBC + 3-(3,4-dichlorophenyl)-1,1-dimethylurea (diuron), carbendazim + diuron, carbendazim + OIT or carbendazim + IPBC. The use of silver nanoparticles as a microbiological and mycological protection in coatings can be an alternative to the use of biocides [16].

Many nano-sized substances reveal interesting new properties compared to forms with larger particle diameters. According to the literature, nanosilver has antibacterial and antifungal properties [17]. The antibacterial properties grow accordingly with increasing the total surface area of the nanoparticles and with reducing their unit dimensions. This is due to the potential for greater penetration by the particles when they are smaller in size. The effectiveness of the antibacterial properties of nanosilver also depends on the particles' shape. Triangular particles show the strongest effects [17-19]. The biocidal effects of silver nanoparticles involve binding to thiol groups located in proteins in bacterial cell membranes, which leads to an increase in membrane permeability and, consequently, to the death of the bacteria [20]. Nanoparticles less than 25 nm in diameter exhibited a MIC (Minimal inhibitory concentration) equal to 6.75-54 µg/cm³, while particles 25 nm in diameter show a MIC in the range of 16.9-13.5 µg/cm³ in relation to multiple drug-resistant bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA) and *Staphylococcus epidermidis* (MRSE), as well as vancomycin-resistant *Enterococcus faecium* [21-25].

According to the varying degrees of effectiveness of the antimicrobial properties of nanosilver obtained via different technologies and the need to recognize the application in the coating field, researches were undertaken whose aim was to evaluate the antifungal activity of silicon paint, plaster and ground plaster containing silver nanoparticles as well as perform a mycological efficacy resistance analysis of tested preparations in relation to different concentrations of nanostructured silver. The use of nanoparticles with antimicrobial properties in construction materials can bring numerous potential advantages, such as reinforcing their hygienic properties, preventing microbial growth and maintaining their mechanical properties.

Materials and Methods

The usefulness of silver nanoparticles for bio stabilising coatings against fungi was evaluated for waterborne products that are more susceptible to fungal growth compared to products manufactured on the basis of organic solvents. Ground plaster with silver nanoparticles and the biocide ACTICIDE[®] MBS, silicone plaster with silver nanoparticles and ACTICIDE[®] MBS, and silicone paint with silver nanoparticles and ACTICIDE[®] MBS were used in the analysis. Silicone paint containing a protective system in the form of a mixture of silver nanoparticles and the ACTICIDE[®] MBF 50 biocide, as well as silver nanoparticles and the ACTICIDE[®] EPW biocide, in their shells were also examined. Data concerning the tested systems are listed in Table 1.

ACTICIDE[®] MBF 50 and ACTICIDE[®] MBS are mixtures of MIT (methylisothiazolinone) and BIT (benzisothiazolinone). The composition of ACTICIDE[®] MBF 50 is also based on the formaldehyde donor, TMAD (tetra (hydroxymethyl) acetylenediurea). These biocides have very high efficiency of protection across a wide spectrum of aqueous formulations. ACTICIDE[®] EPW is a biocide coating formulated from a mixture of a modified algicide, Diuron, in combination with fungicides. Such coatings are used to protect paint, plaster, adhesives, etc.

Preparation and properties of suspended silver nanoparticles

A suspension of silver nanoparticles (500 ppm) was obtained by chemical reduction of Ag⁺ ions in an aqueous environment. Silver nitrate (V) (Sigma Aldrich, 99.90-99.99%) was applied as a source of silver ions. An aqueous extract of dried raspberries was used as the source of chemical compounds having reducing and stabilizing properties. Sodium nitrate at a concentration of 0.01 mol/dm³ was used as the pH regulator.

Sample number	Type of coating	Active substance	Evaluation of fungal growth (final readings after 21 and 28 days of incubation)		
			Repetitions	Average	The criterion for acceptance: <4 according to the scale (growth on up to 50% of the sample surface)
1	Uni Grunt	silver nanoparticles (1%)*	2; 4; 4; 4; 3; 3	3.3	positive
2	Uni Grunt	ACTICIDE [®] MBS	1; 2; 0; 0; 0; 1; 1; 3	1	positive
3	Silicone plaster	silver nanoparticles (1%)*	1; 1; 1; 1; 1	1	positive
4	Silicone plaster	ACTICIDE [®] MBS	0; 0; 0; 0; 0	0	positive
5	Silicone paint	silver nanoparticles (1%)*	3; 3; 3; 3; 3	3.0	positive
6	Silicone paint	ACTICIDE [®] MBS	3; 2; 2; 3; 3	2.6	positive
7	Silicone paint	silver nanoparticles (2%)*	1; 1; 2; 2	1.5	positive
8	Silicone paint	silver nanoparticles (3%)*	2; 2; 2; 3	2.3	positive
9	Silicone paint	silver nanoparticles (1 %)* + ACTICIDE [®] MBF 50 "in can" (0.05%)	1; 3; 3	2.3	positive
10	Silicone paint	silver nanoparticles (1%)* + ACTICIDE [®] EPW (0.1%)	0; 0; 0; 0; 0	0	positive
Final evaluation					Criterion fulfilled

Table 1: Mycological efficacy results.

A solution of chemical compounds having reducing and stabilizing properties was obtained from dried raspberries by leaching them in an aqueous phase. For this purpose, 4.5 g of dried raspberries and 500 cm³ of water were introduced into a round bottom flask with a reflux condenser. The flask was placed in a water bath (30°C). The content was vigorously stirred for 5 min. After this time the contents of the flask were filtered and the filtrate was retained.

In order to obtain a silver nanoparticle suspension, 20 cm³ of raspberry extract was added to 480 cm³ of an aqueous solution of silver nitrate (V) (0.0048 mol/dm³) and the pH was adjusted to 10 using the NaOH solution. The mixture was stirred on a magnetic stirrer for 5 min. The contents of the round bottom flask were transferred to a plastic container and allowed to stand at room temperature for 120 hours. The resulting suspension of silver nanoparticles was analysed using a UV-Vis spectrophotometer (UV1800 Rayleigh). The average particle size and zeta potential, ξ , were determined by a dynamic light scattering method (DLS, Zetasizer Nano ZS Malvern Instruments Ltd.).

Characteristics of the nanosilver suspension

The absorption spectra of nanosilver suspensions in the process of aging are shown in Figure 1. The peak at 400-450 nm corresponds to the characteristic surface plasmon resonance of silver nanoparticles. Surface plasmons are compatible oscillations of valence electrons of atoms on the material's surface. The absorption of radiation by metallic nanoparticles depends essentially on their size and shape. The plasmon band is not symmetrical, which means that the solutions contain aggregated particles. This is confirmed by the size distribution of the nanoparticles, which is presented in Figure 2. As a result of the process, silver nanoparticles with an average particle size of 57 nm and a ξ potential = -20 mV were obtained.

Mycological efficacy studies

Tests of resistance to fungi were carried out according to EN 15457:2007 [26]. Test samples (about 2 g) were applied once with a Drigalski spatula on sterile filtration filters with a pore size of 0.45 microns so as to form a coating, which was later conditioned for 5 days at 23 ± 2°C at a relative humidity of 50 ± 5%, according to EN 23270 [27]. Samples prepared in replicates were applied to the surface of malt medium (3%) and agar (1.5%). To determine the antifungal activity of the dry coating, each of the samples was inoculated with

0.2 cm³ of a mixture of a previously prepared suspension containing, in equal proportions in a total 10⁶-10⁷/cm³, spores prone to fouling in the external environment – *Cladosporiumclado sporoides* ATCC 16022 and *Alternaria alternata* DSM 62010, with others prone to fouling under internal conditions – *Rhodotorulam ucilaginoso* ATCC 66034, *Aspergillus Niger* ATCC 16888 and *Geotrichium candidum* ATCC 34614. The samples were incubated at 24 ± 2°C for 21 days in accordance with the methodology of the research. Evaluation of the activity of the nanosilver in the test sample was based on a control test sample prepared containing biocides (Table 1). In accordance with the test method, the effectiveness of the coating was considered satisfactory if fungal growth occupied less than 50% of a tested sample - grade 3 according to the scale of the standard [26]:

0 - no fungus on the sample surface,

1 - mycelium growth on up to 10% of the surface,

2 - mycelium growth on greater than 10% but not more than 30% of the sample surface,

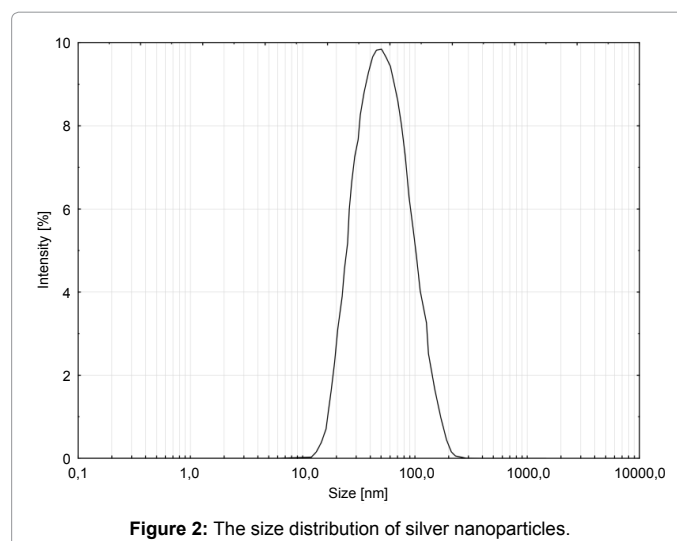
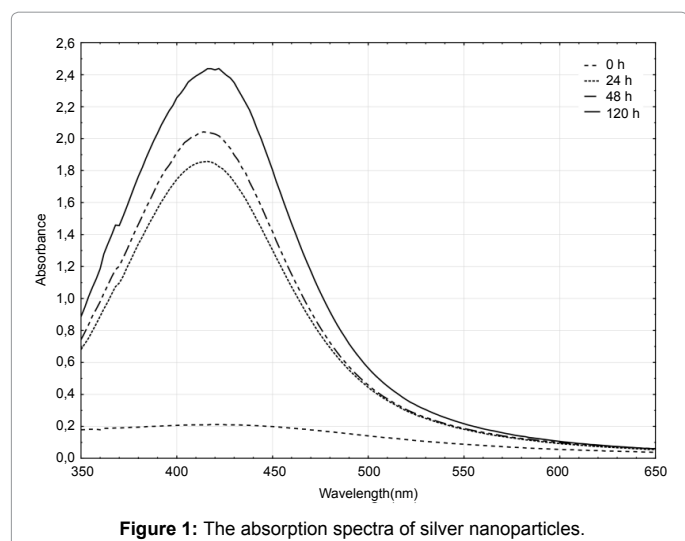
3 - mycelium growth on greater than 30% but not more than 50% of the sample surface,

4 - mycelium growth on greater than 50% of the sample surface.

Results and Discussion

All tested samples containing silver nanoparticles showed effective mycological resistance, comparable with the samples protected by biocides, garnering positive final assessments according to the scale given in the EN 15457:2007 standard [26] (Table 1 and Figure 3).

The soil sample containing 1% silver nanoparticles (mycelium growth greater than 30% but not more than 50% of the sample surface) received a grade of 3, while the ground plaster with ACTICIDE® MBS biocide was rated grade 1 (mycelium growth on up to 10% of the surface). There was no difference in the protection against fungi between silicone paint with a 1% content of silver nanoparticles and silicone paint with ACTICIDE® MBS because both samples were rated grade 3. Silicone paint with either a 2% or 3% silver nanoparticle content was characterized by very good efficacy of protection (grade 2), exceeding that of silicone paint with ACTICIDE® MBS biocide (grade 3). The most satisfactory results were noted for plaster silicone with 1%



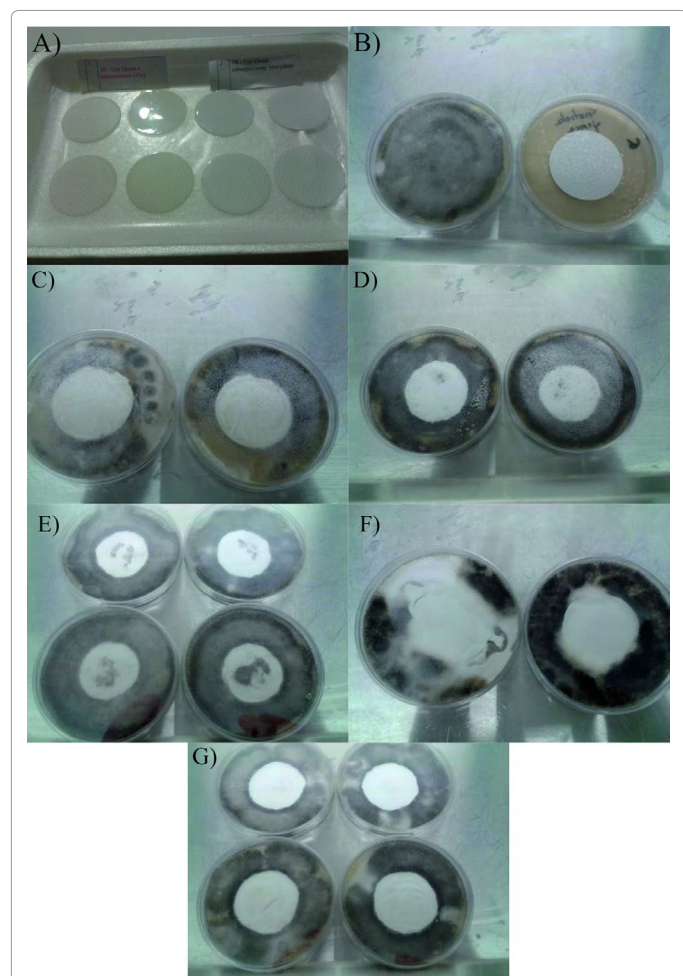


Figure 3: Photographs showing the samples analysed: A) prepared samples, B) positive and negative controls for fungal growth, C) silicone paint with nanosilver (2%), growth <10%, D) silicone paint with nanosilver (2%), growth between 10% and 30 %, E) silicone paint with nanosilver (3%), growth from 10% to 30% and between 30% and 50%, F) silicone paint protected by ACTICIDE® MBS biocide, growth between 30% and 50% and between 10% and 30%, G) 100% inhibition of fungal growth after 21 days with silicone plaster protected by a coating with nanosilver (1%) and EPW (0.1%).

silver nanoparticles, for plaster silicone with ACTICIDE® MBS and for the system with 1% silver nanoparticles and 0.1% ACTICIDE® EPW, whose evaluations were, respectively: 1, 0, and 0.

According to the requirements [27], the positive control for fungal growth showed an increase from 50% to 100% on the 7th day of the studies, getting a grade of 4, which was the basis for reading the results at this time. However, in order to check the stability of the results obtained, the study was continued out to 21 days (optional conditions compatible with the standard). The results were stable for all samples except the sample of silicon paint with 1% silver nanoparticles and 0.05% ACTICIDE® MBF 50, for which the assessment deteriorated from 2 to 3 on the adopted scale but still met the requirements for an agent with antifungal properties. Additional readings were performed to confirm the stability after 28 days and they were consistent with readings after 21 days.

In view of this little recognized research area, the results can be referenced only to general research in this area and MIC and MBC values obtained for silver nanoparticles. Medical studies have reported

that nanosilver exhibits high antifungal activity against *Candida spp* at a concentration of about 1 mg/dm³. Silver nanoparticles have fungicidal and fungi static effects on dermatophyte *Trichophyton mentagrophytes* and *Candida spp* [28-31]. There are also reports of the antifungal activity of the obtained silver nanoparticles during maintenance of materials for the production of footwear, wherein the 1% solution inhibited the growth of most strains of yeast-like fungi and moulds [30]. The recommended concentration of aqueous solutions containing nanostructured silver is 45 ppm. It acts as a biocide for 94% of the microorganisms isolated in museums and archives [6].

Conclusion

The antifungal activity of nanosilver in the checked products tested in accordance with EN 15457:2007 [26] was confirmed. The present study demonstrated the effectiveness of silver nanoparticles at a concentration of 1-3%, corresponding to 5-15 ppm nanosilver.

The obtained protection matched or exceeded the results achieved by applying active fungicidal substances as biocides. The use of silver nanoparticles at a concentration of 2% or 3% in the silicone paint is particularly noteworthy, because the efficiency of its use outperformed the known biocide. The greatest efficiency of the protective coating was noted in a mixed system: silver nanoparticles (1%) and ACTICIDE® EPW (0.1%) achieved 100% inhibition of tested fungi growth (Figure 3).

Nanostructured silver may be a promising alternative to currently used biocides.

The purpose of eco-labelling is to promote products that have limited impact on the environment. Pursuant to Regulation WE No. 1980/2000, products must meet certain criteria for facade paints that are given in the British Standard BS 3900-G6:1989 "Methods of testing for paints. Assessment of resistance to fungal growth". The question remains whether the tested nanostructured silver also provides the required protective efficacy against fungi for external coatings. There is also a need to check the antibacterial activity of the tested nanosilver, in particular resistant strains, which will be the subject of further studies.

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