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INFLUENCE OF NANOTECHNOLOGY FOR THE TREATMENT AND PROTECTION OF NATURAL STONE AGAINST BIODETERIORATION

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AgNPs have various of applications due to their special properties such as the strong antimicrobial and antifungal activity, including to combat biodeterioration on various surfaces. Using various siloxane-based coupling agents, which, due to their chemical structure, can react with both the treated surface and AgNPs, anchoring themselves onto the surface to be treated, can act against microorganisms and biofilm formation. Depending on the type of coupling agent, AgNPs can fix onto the treated substrate or even penetrate inside the surface, thereby achieving better efficacy against microorganisms. The type of AgNPs is crucial, not just the concentration but also the type which is used. This study provides a comparative analysis between substrates treated with silanization agents at a concentration of 100% and those treated with a mixture using various laboratory obtained AgNPs.

Keywords: silanization marble stone surfaces, antimicrobial effect of AgNPs, protection marble stone surface against biodeterioration

1. Introduction

Nanoparticles, characterized by their small sizes and large contact surface exhibit unique properties that render them suitable for multiple applications in various fields [1, 2]. A notable enhancement in properties is observed in NPs due to a heightened ratio of surface area to volume in comparison to their corresponding bulk materials. Application of NPs in various sectors as water waste treatment, solar

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cells and batteries in agriculture as treatment for plants, in electronics, medicine are controlled by their efficiency which are influenced by the type of synthesis [3]. Silver nanoparticles, due to their unique properties and interactions at the nanoscale, have demonstrated promising effects when incorporated into stone treatment processes, particularly for materials like marble. AgNPs are well known with high antibacterial effect, this property helps to apply them on stone treatment with high interest in the field of natural surface enhancement and preservation [4-7].

The bactericidal activity of AgNPs involves intricate processes, wherein damage is inflicted upon bacterial cell walls and plasma membranes, concurrently hindering DNA replication and protein synthesis. The effect primarily stems from gradual release of Ag^+, thereby facilitating their biocidal efficacy at lower concentrations compared to silver salts [8]. One of the main factor which cause and affect the aspect the structure and resistance of the stone is biodeterioration [9]. AgNPs due to their antibacterial and antifungal effects can be applied in stone protection and further research is necessary for fully understanding the interaction in time between stones and protection agent, and potential environmental implications.

Biodeterioration constitutes an undesirable phenomena induced by microorganisms, encompassing the progressive deterioration and degradation of materials resulting from the metabolic activities of living organisms, including bacteria, fungi, algae, and lichens [9-12]. In the context of stone, biodeterioration can induce chemical, physical and aesthetic detriments[13]. Over time, microorganisms can progress to form biofilms rendering them more resilient by virtue of their integration into an organic matrix firmly fixed to the stone structure. The biofilm formed on the structure of stone can trap moisture and accelerate weathering process [14, 15]. Biodeterioration can have aesthetic impacts such as discoloration, staining which affect the aspect of stone but also severe degradation. Environmental factors have an important influence on both the extent and pace of biodeterioration. Humidity, temperature and sunlight exposure collectively are main factors in modulating the growth and metabolic activity of microorganisms thereby shaping development of biodeteriorative processes.

The protection of stone surface, conservation of monuments and claddings is an important issue requires a multidisciplinary approach based on microbiology, chemistry and physics [16, 17]. Removal biofilms operations, by cleaning is usually performed using mechanical or chemical techniques, followed by a protection treatment [18] or impregnation operation with various products starting with from biocides, different siloxanes or to use special components of biofilm formation itself as treatment.

Silver nanoparticles (AgNPs) can be incorporated into protective coatings and applied to stone surface by pure physical adsorption or by chemical approaches, by using coupling agents for functionalized surfaces for bonding silver nanoparticles. The siloxanes referred to as functionalization products serves as intermediary compounds during the impregnation process owing to their molecular structure (Fig. 1) [19-21]. Those siloxanes with thiol groups as reactive group can form with silver nanoparticles strong covalent bonds due to the affinity between thiol groups and silver nanoparticles [22-24]. These interactions provide a strong attachment to the substrate and enhance the stability of antimicrobial layer.

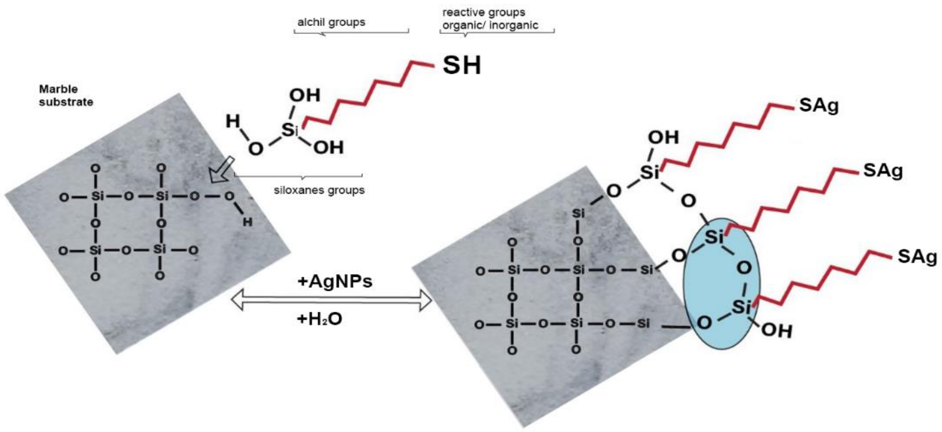


Fig. 1. Mechanism of action siloxanes coupling agent on stone substrates

The current study investigates the impact of laboratory-synthesized silver nanoparticles, obtained [25], through chemical reduction, when applied to the marble stone substrates. The marble substrate is treated before with a siloxane-based as a coupling agent. Laboratory derived AgNPs are applied, by brushing, to the siloxane treated marble surface [25] aiming to achieve enduring protection against the formation of biofilm [26-28]. Thus utilization of AgNPs in conjunction with the functionalization agent operates through different mechanisms to impede the formation of microorganisms and prevent biofilm development beyond substrate protection and preservation of its quality aspect.

2. Materials and methods

2.1 Materials

AgNPs used in this work were synthetized in laboratory based on chemical synthesis previously published [25]. The samples were selected based on their antibacterial efficacy. Based on antimicrobial tests the selected samples for tests are those obtained by wet chemical routes, at room temperature, coded in this work with sample 1 (coded AgNPs s1) and sample 2 (coded AgNPs s2) and samples obtained at temperature, by solvothermal route coded with sample 3 (coded

AgNPs s3). These colloidal AgNPs solutions, of different concentration (10 ppm, 100 ppm or 1000 ppm) were used and revealed in the table (

Table 1). The marble was chosen based on information related to the material usually used for monuments, architecture pieces or historical claddings both interior or exterior pavements, staircases, columns, etc. Marble stone is a metamorphic rock formed from recrystallization of limestone from dolomite under high pressure and temperature conditions. Chemical composition of marble are carbonate minerals, mainly based on calcite and dolomite and composition can vary depending impurities [29]. The XRD image (Fig. 2) show the marble sample contains crystallized calcite ($CaCO_3$) and no additional crystalline phase being identified.

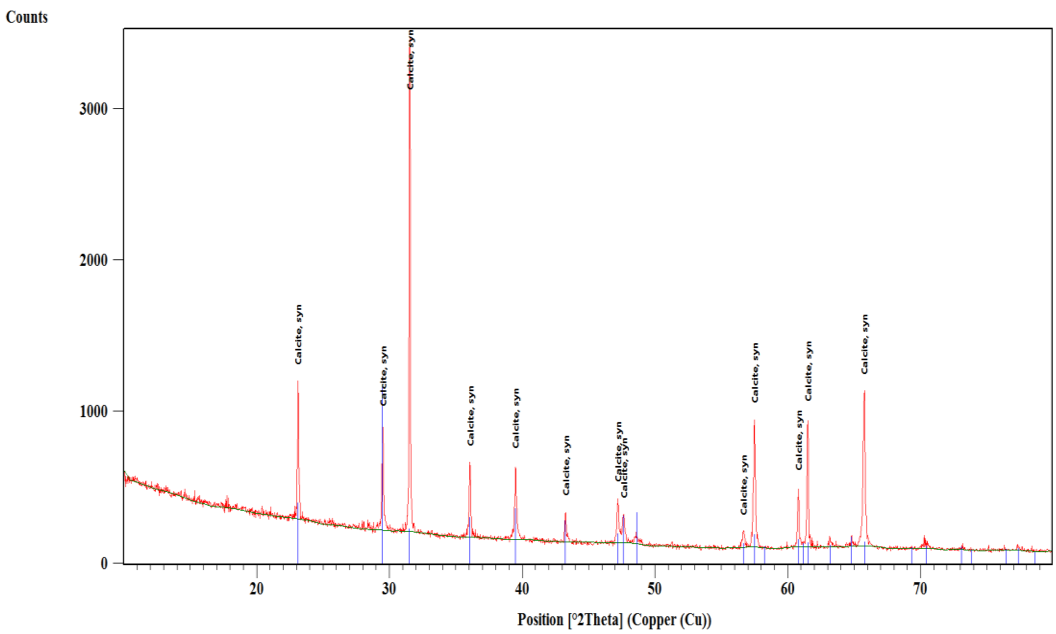


Fig. 2. XRD pattern of the marble (control)

2.2 AgNPs used for treatment and protection of marble surfaces

Silver nanoparticles were synthesized employing two distinct chemical methods: the conventional method at room temperature and the solvothermal method [25]. Based on the antimicrobial outcomes figured in the table (Table 1) , the solution produced through the conventional reduction method at room temperature at 10ppm, 100 ppm alongside the solution derived from the solvothermal synthesis route at 1000 ppm were selected further analysis [25].

Table 1

Type of AgNPs solutions used in tests, characterization

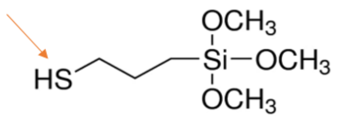
Code of AgNPs	**Type of synthesis method**	**Concentration of AgNPs**	**Properties of AgNPs**
AgNPs s1	Conventional reduction synthesis at room temperature using two reduction agents	10 ppm	small sizes of nanoparticles, less 10 nm sizes, with different shapes
AgNPs s2	Conventional reduction at room temperature, using one reduction agent	100 ppm	small and medium sizes, 20-30 nm, with different shapes, mainly truncated and triangular AgNPs shapes
AgNPs s3	Solvothermal method at 260 °C	1000 ppm	spherical shapes and large sizes, 100 nm

2.3. Modification of the stone surface with functional groups

The stone samples utilized were prepared with dimensions of 1x1x1 cm^3 or 1x1x0.1 cm^3, depending on the subsequent evaluation techniques. The marble stone samples were cleaned before treatment by washing with warm water and bicarbonate solution followed by rinsing with deionized water and subsequently dried in an oven at 100°C for 24 hours. A bicarbonate solution is employed to cleanse the stone surface maintaining a neutral environment pH (pH=7). The marble stones treated, in the first step, with 3 MPTMS (3 mercaptopropyl methoxysilane from Aldrich, a siloxane coupling agent (Table 2) with –SH (thiol) functional group. Using specific siloxanes with thiol groups can leading to a stable covalent bonds by which thioalkyl groups are attached onto the surface of the stone [30] (Fig. 1). The organic groups featuring –SH end groups (Table 2) remain positioned on the outer side of the stone surface, imparting novell properties, notably a heightened affinity for AgNPs.

Table 2

Type of coupling (functionalization) agent used for treated stone

Siloxanes - coupling agents	Formula
3 mercapto propyl trimethoxysilane (3 MPTMS)	

Importantly, this functionalization does not obstruct the stone's pores allowing it to breathe [31]. In the initial phase, marble samples were treated with the functionalization agent in two variants i) 100% functionalization agent ii) 50%

functionalization agent+ 50% i-propanol (IPA). The incorporation of i-propanol aims to reduce the viscosity facilitating improved penetration into the surface structure (pores and cracks). The impregnation process was executed via brushing to simulate practical working condition.

2.4. Characterization of the obtained AgNPs

FTIR imaging was performed using a Thermo IN50 MX microscope (Thermo Scientific). FTIR imaging was utilized to gather information regarding both the stone structure and the presence of siloxane products (functionalization) on the treated surface. The FTIR microscope analysis was conducted in reflection mode, employing germanium ATR unit to scrutinize their structural features.

The surface morphology of the samples was investigated via a QUANTA INSPECT F electron microscope equipped with a field emission gun providing a resolution of 1,2 nm along with an energy - dispersive (EDS) detector featuring 133 eV MnK resolution was employed to examine samples coated with carbon. Images were obtained by capturing backscattered electrons (BSE) to emphasize variations between elements with high atomic weight (silver) and lighter elements (Si) Additionally, secondary electrons were collected using an in-lens detector, particularly at high magnifications. SEM was conducted on the impregnated stone samples to scrutinize the surface morphology. The semi-quantitative elemental composition was assessed through Energy Dispersive X-Ray Spectroscopy (EDS), and the uniformity of the modification was tracked via EDS mapping.

The UV–Vis spectra were recorded using an Evolution 300 UV- VIS spectrophotometer in absorbance mode (190-1100 nm), selecting 1nm data interval, 2nm bandwidth and 240 nm/min scan speed. All samples were recorded within 10 mm quartz cuvettes, at ambient temperature.

X-Ray diffraction experiments were executed utilizing the PANalytical Empyrean equipment, operated at 45kV and 40mA in Bragg-Bretanno geometry employing CuK α radiation (λ=1.5418Å). The diffractometer is equipped with a fixed 1/4° divergent slit, 0.02°Soller slit and 1/2°anti-scatter slit on the incident beam side. On the diffracted beam side there is a 1/2°anti-scatter slit, 0.02°Soller slit, and 0.02 mm Ni filter mounted on PIXCel3 D detector.

2.5. Antimicrobial evaluation

The antimicrobial activity was tested by different type of stone substrate using AgNPs synthetized in our laboratory and siloxanes as coupling agents. The antimicrobial efficacy of the applied AgNPs on marble substrates was assessed against the gram-positive bacterial strain *B Subtilis*, which is commonly encountered on such surfaces.

2.5.1 Biofilm inhibition

Analyzing the antibiofilm effect of silver nanoparticle by counting the colonies developed on a medium. For this method, 24 wells have been prepared for conducting planktonic culture analyses. However, this time, the samples that have been in the plate wells throughout the entire incubation period will be used. The same procedure has been applied both for the 24-hour plate and the 48-hour plate. After 24 hours of incubation, the silver nanoparticle was extracted using 1 ml of sterile physiological water (SPW) to remove non-adherent microbial colonies. Subsequently, they were placed in separate Eppendorf tubes along with 1ml of SPW. The tubes were vortexed for 30 seconds to detach adhered microbial cells and incorporate those from the biofilm formed on the stones' surface, aiming to quantify viable colonies that would subsequently develop on culture media. Culture plates, inoculated with microbial suspensions containing cells detached from the biofilms formed on the dressings for 24 hours, were subsequently incubated for an additional 24 hours at 37°C. After incubation. Following the incubation period, the plates were examined for quantification colony-forming units (CFU/mL). The same method was applied for testing of marble samples treated with AgNPs and exposed outside.

3. Results and discussions

3.1 FTIR spectroscopy

The graphic FTIR images for marble stone treated with the coupling –agent in both variants, comparing with marble control, were performed for detecting the presence of coupling agent (**Fig. 3**).

The FTIR spectra of the applied treatments reveal the characteristic stretching vibrations corresponding to peaks of calcium carbonate ($CaCO_3$) in the range of 1430-1500 cm^{-1}, 880-720 cm^{-1}. This, coupled with the XRD analysis (Fig. 2) confirm the presence of calcite as the primary component in both the untreated marble and functionalized marble samples.

The bands observed at approximately 1430 and 880 cm^{-1} can be ascribed to carbonate anions originating from calcite. In both instances, these spectral features arise as a result of the surface treatment with 100% 3MPTMS (**Fig. 3**) and mixture of 3MPTMS and IPA (**Fig. 3** c) the presence of siloxanes groups can be identified on the marble surface proving their functionalization. These siloxane groups can be identified in the 1100-1000 cm^{-1} range [32] marked in the rectangular shape (**Fig. 3**) [33-35]. Based on the FTIR spectra (**Fig. 3**) because of the quite same intensity of the peaks, it can assume that the surface functionalization is not altered by dilution with solvent isopropyl alcohol (IPA).

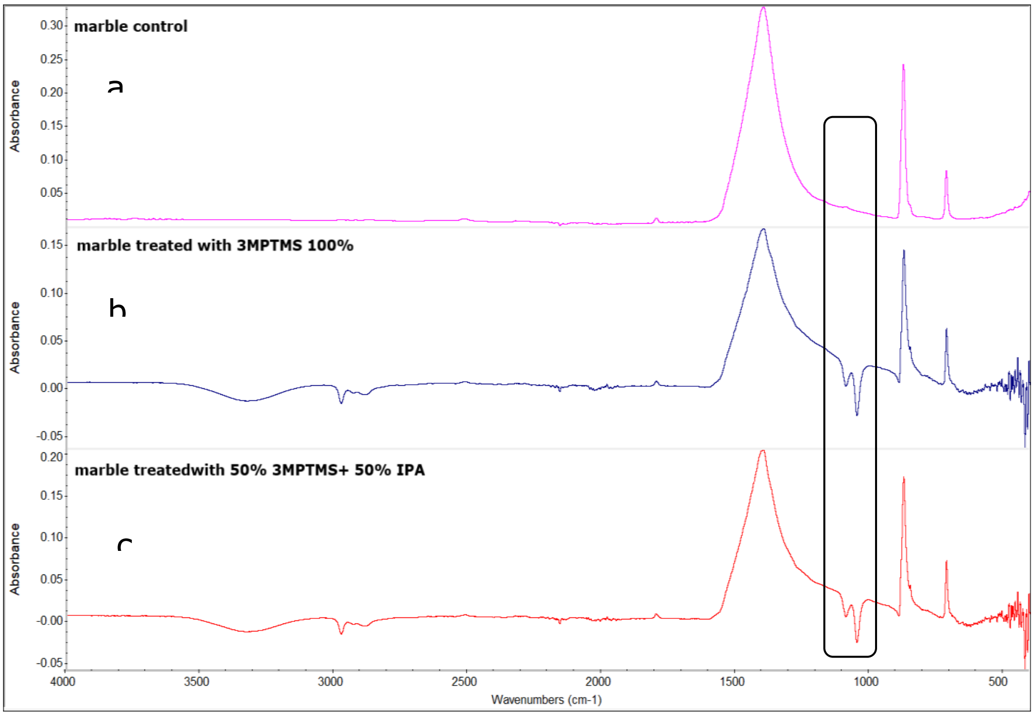


Fig. 3. Comparative FTIR images of marble samples (a) or the samples treated with 100% 3MPTMS (b) and the mixing 50% 3MPTMS +50% IPA (c)

The IR maps for the marble surfaces modified with pure siloxanes agent or with a mixture of siloxanes agent and IPA are monitored at ~1430cm^{-1} corresponding to carbonate and ~1050cm^{-1} corresponding to the silicate band (Fig. 4). The differences which are observed are due to the natural variation in the composition and microstructure of the natural materials, and thus, these differences are leading to differential adsorption of the functionalization to the surface. The presence of siloxane on the surface is indicated by the peaks value around 1100 – 1000 cm^{-1} corresponding to Si-O-Si (Fig. 4 a,b), confirming the presence of the coupling agent on the surface of stone. Comparing the IR maps for the two selected wavelengths it can see that the treatment with the pure 3MPTMS lead to a stronger difference between the two maps recorded at 1430cm^{-1} (carbonate – associated with the marble) and 1050cm^{-1} (silicate - associated with the coating) because this agent is not well penetrating inside the pores and cracks of the marble. In case of treatment with diluted 3MPTMS, the IR maps shown a relative better similarity because the dilution with alcohol improves the marble stone penetration (Fig. 4).

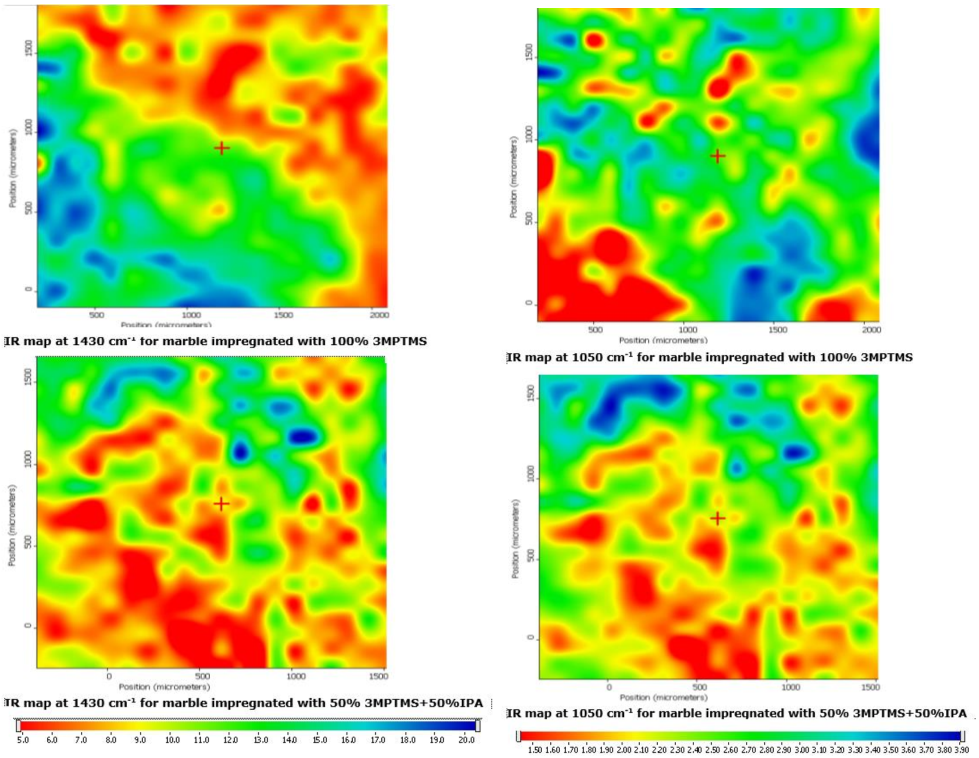


Fig. 4. IR maps of marble at the wavelength 1430 cm^{-1}(a) and 1050 cm^{-1} (b) treated with 3MPTMS functionalization coupling agent

3.2 Scanning Electron Microscopy (SEM)

SEM images of untreated and treated samples provide information related to the aspect of surfaces. This technique gives the aspect of coupling agent in the relationship with treated substrate [36]. The obtained SEM images show the untreated surface (control) a compact surface with fine grains of calcite minerals, closely packed crystals which confer a stable structure (**Fig. 5** a). The treated surface with 3MPTMS show an aspect uniform, smooth surface, as silane coupling agent cover fine grains and porosity of stone (**Fig. 5** b). Comparing SEM images providing from the two types of stones can consider that depending of the structure stones should choose the certain coupling agent [30, 37-40]. The coupling agent used to show that formed a film coating obtained a relative smooth surface. Depending on the type of coupling agent it can form a film as a crust or can penetrate easily the surface of them [14, 41-43]. Given these observations a more in-depth analysis was conducted on the synergistic effect of the coupling agent along with AgNPs which imparts a resilient surface resistant to biofilm formation.

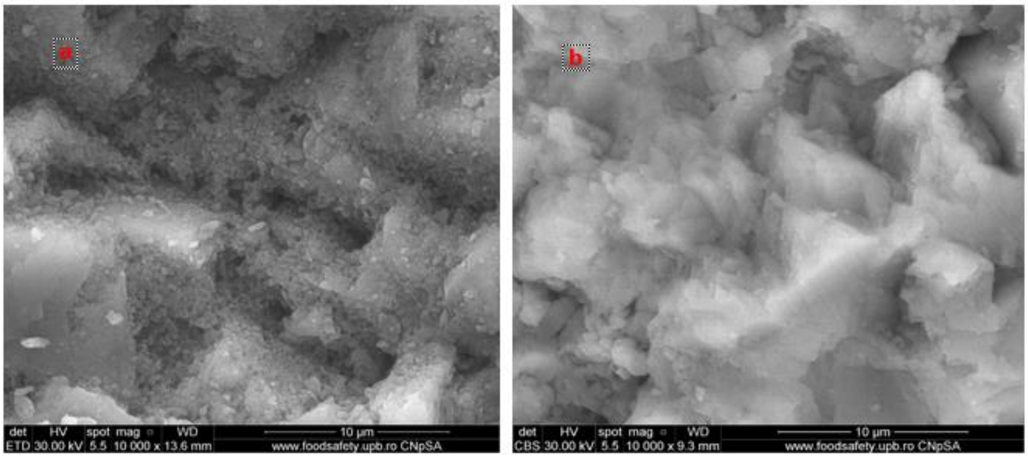


Fig. 5. SEM images a) marble control; b) marble treated with 3MPTMS

Based on scanning electron microscope images, whether acquired through BSE or SE as well as EDS, the discernible presence of Ag NPs on marble treated with the siloxane agent and subsequently treated with additional AgNPs cannot be emphasized. This fact is due to the low concentration of AgNPs as well as due to the partial penetration of AgNPs inside the porous structure.

3.3 UV-Vis quantified of AgNPs adsorption

In the light of the necessity to quantify the absorbed amount of AgNPs, UV-VIS assessments were employed. The absorption of AgNPs was determined by comparing the initial and the final absorbance values of tested solutions, for each type of AgNPs used for the treatment of the stones. Despite the inability of SEM images to definitively establish the presence of AgNPs on the stone surface UV-VIS absorption analysis demonstrated effective absortion of AgNPs to the treated surface as revealed in figure (Fig. 6). The UV-VIS spectra indicate that the absorption characteristics of both AgNPs denoted s1 and s2 and synthetized at room temperature are notably influenced by both substrate’s nature and the functionalization agent. The absorption of AgNPs s1 and s2 is relatively high. Both graphics (Fig. 6) corresponding to AgNPs s1 and s2 after samples were immersed in AgNPs show no peak in case of AgNPs s2 indicate totally absorption and for AgNPs s1 indicate more 50% absorption. The functionalisation with 3-MPTMS, for AgNPs obtained by solvothermal method (s3), is not leading to a total absorption of AgNPs. It is noticed that the AgNPs s3 sample with 1000 ppm concentration shown an absorption of about 25% on the marble substrate treated with 3MPTMS which means that the absolute AgNPs retention is higher comparing with the previous cases (Fig. 6). The absorption of AgNPs on functionalization surface can be influenced not only the concentration but the sizes and shapes of nanoparticles [44].

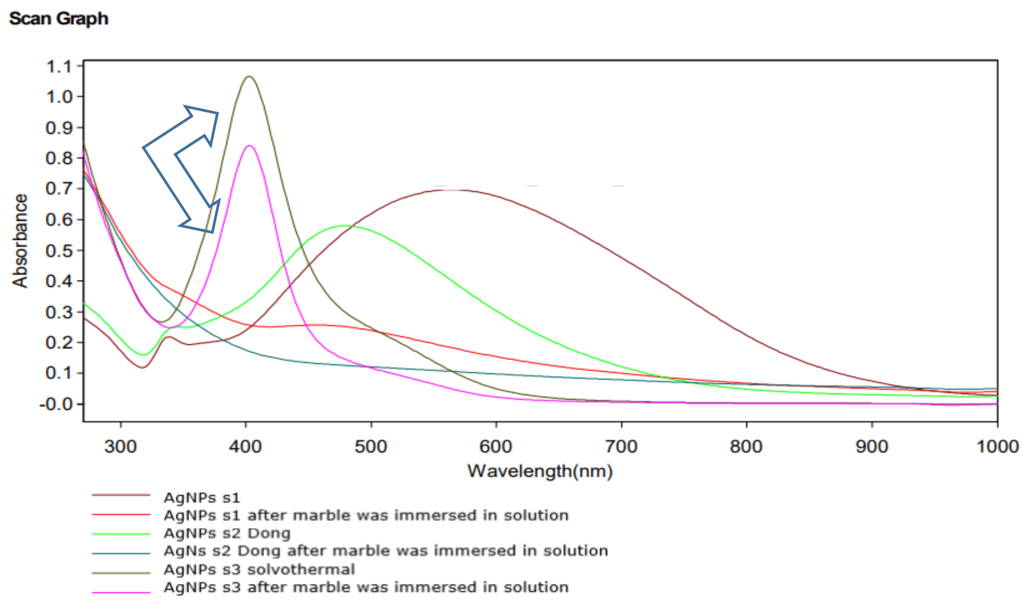


Fig. 6. The quantitative determination of the presence of AgNPs on the treated stone conducted by UV-Vis spectroscopy

3.4. Assessment of antimicrobial activity

The antimicrobial evaluation was performed on marble samples, measuring 1x1x0,1 cm in accordance with the established standard protocol. The analysis of the stone substrate impregnated with variant *i* (3MPTMS 100%) and variant *ii* (50% 3MPTMS+50% IPA) and treated with AgNPs with various concentration reveals that all samples the exhibited resistance compared to sample control (Fig. 7). Specifically, the treated marble substrate, functionalization with 3 MPTMS and AgNPs (Fig. 7) displayed a notable decrease in biofilm resistance, showing approximately by 2,5 values in logarithmic scale for sample treated with AgNPs s3 (Fig. 7) comparing with sample control. The graphic of adherence and biofilm (Fig. 7) show that the sample treated with AgNPs s3 has better efficiency comparing with sample AgNPs obtained at room temperature and less concentration. On the other hand, comparing samples treated with mixing (50% 3MPTMS+50% i-propanol) show better efficiency samples s1 and s2 against *B subtilis*. Probably, the solvent aids in the impregnation of the surface and into depth, enabling small-sized AgNPs to penetrate easily, thus potentially exerting antimicrobial action both on the surface and from within. Practically, the efficiency of surfaces treated with AgNPs s1 and s2 are with 4 log units less the marble control (Fig. 8) and 3 log units less for AgNPs s3. The treated surface with different concentration of silanes coupling agent has influence at the AgNPs efficiency.

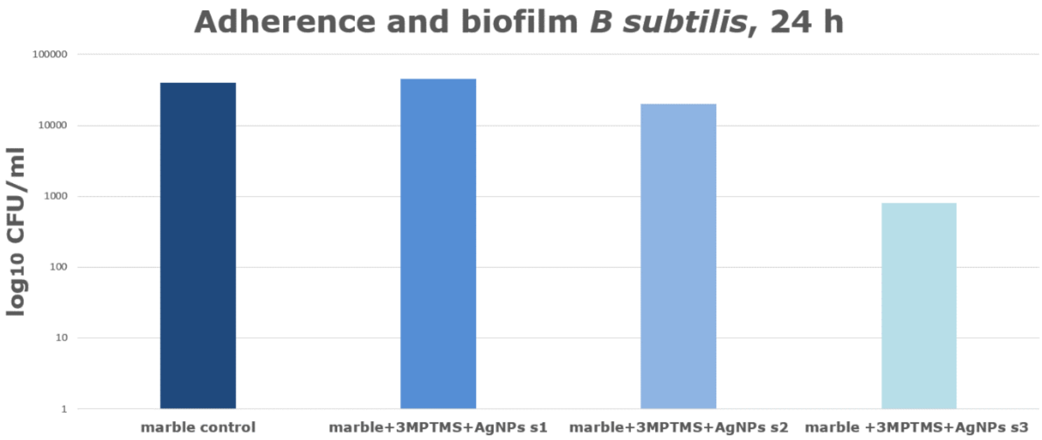


Fig. 7. Adherence biofilm of *B Subtilis* treated marble samples with 100% 3 MPTMS

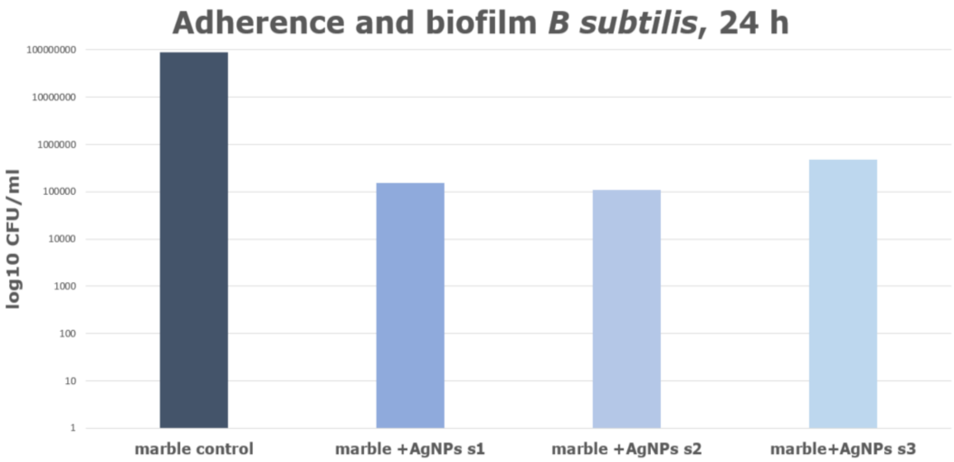


Fig. 8. Adherence and biofilm of marble samples treated with mixing (50% 3MPTMS+50% IPA) and various AgNPs concentration

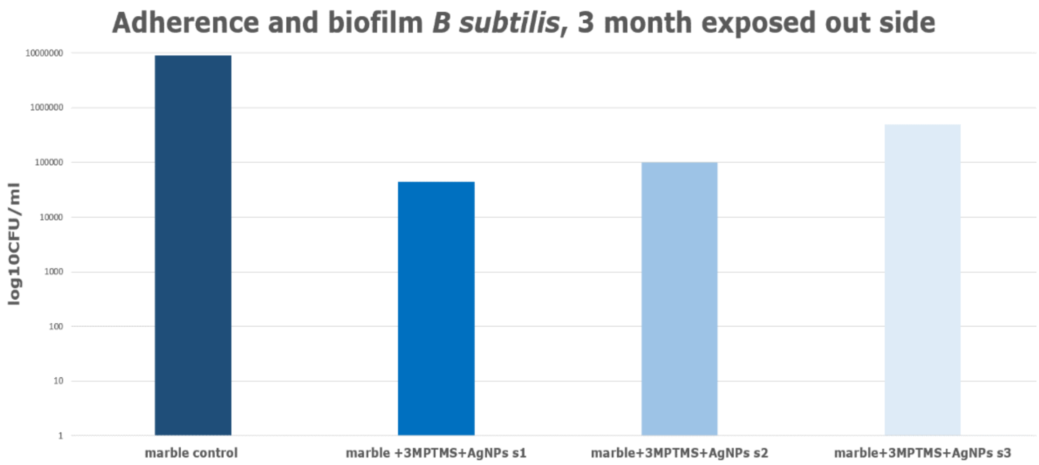


Fig. 9. Adherence and biofilm marble samples treated with various AgNPs exposed outside

The analysis of *B subtilis* biofilm adherence on marble substrate with various concentration of AgNPs treated with 3MPTMS 100%, exposed outside for 3 month, show efficiency against *B subtillis* (Fig. 9) for all samples, better activity for AgNPs s1 with 10 ppm concentration with various shapes, mainly truncated. The test made in laboratory (Fig. 7) with 3 MPTS 100% used show, for 24 h, a lack of efficiency for AgNPs 10 ppm, comparing with marble control. Comparing results obtained for samples exposed outside (Fig. 9) AgNPs s1 show better efficiency comparing with marble control and the rest of AgNPs samples (Fig. 9). This difference can be explained considering the following aspects: *i*) truncated triangular nanoparticles are more efficient comparing the nanoparticles with other shapes and, *ii*) on short term, the binding of the nanoparticles by the means of SH groups make it unavailable for short term activity (24h) but protect very well on long term, such as 3 months.

4. Conclusions

The impregnation operation with coupling agent in 100% or mixing with iso-propanol definitely influences the efficiency of AgNPs against of B subtilis. Practically, the mixture with alcohol helps better penetration of substrate for high concentration and larger nanoparticles, these leads to laboratory results with better efficiency against *B subtilis*. Regarding the analysis, when only the substrate treated with 3 MPTMS 100% and exposed to the exterior is considered along with variations of AgNPs, it is observed that the nanoparticles with low concentration and small size with truncated shape are favorable, showing better efficiency. It is worth discussing and monitoring the action of these same nanoparticles on marble substrates treated with a mixture or with other coupling agents. The effectiveness of nanoparticles action can become synergistic agent if chosen correctly and is crucial in the action against microorganisms. The efficiency of AgNPs onto stone surfaces is not depending only on the concentration of AgNPs there are other many factors which lead to the high efficiency against microorganisms. The surface of marble treated with 3MPTMS coupling agent and AgNPs did not exhibit any changes, any coloration in appearance throughout the conducted tests. This study demonstrates that this chemical approach using MPTMS as coupling agent of AgNPs on the surface of stones (marble) is assuring antibiofilm activity in real conditions. Further works will be done to assess the stability and antibiofilm activity over longer period of time, the current study being on a period of 3 months.

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