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Functionalized Ag Nanoparticles for Antimicrobial and Antifungal Protection

ABSTRACT

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Bucharest 2024

Contents

In this abstract, the content and numbering of tables and figures have been preserved in accordance with the original thesis.

I. INTRODUCTION

I.1 Obectives of the doctoral thesis

The aim of this study is to investigate the physicochemical and biological differences between AgNPs synthesized chemically at room temperature and at elevated temperatures, aiming to select those AgNPs suitable for antimicrobial protection. Additionally, the study aims to assess the influence of both the substrate treated with various coupling agents at varying concentrations and the presence of silver nanoparticles with different specifications and concentrations. Multiple types of natural stone substrates were utilized to analyze the antimicrobial effect of AgNPs and the influence of coupling agents on treated substrates. Comparative analyses will be conducted between substrates treated with biocidal coupling agents and substrates treated with coupling agents and various AgNPs to observe efficacy and highlight materials that efficiently act in small quantities with reduced toxicity towards the environment and human health.[1-3]. These analyses will lead to conclusions regarding whether coupling agents (silanization) influence both the treated substrate and the silver nanoparticles used, whether certain specifications of silver nanoparticles lead to better efficacy against biofilm formation, and whether certain substrate types require a special treatment different from those analyzed

II. GENERAL ASPECTS REGARDING NANOPARTICLES

II.1 Generalities about Nanomaterials, Nanoparticles, Nanotechnology

Nanotechnology is the science that deals with achieving nano-sized particles and controlling their shapes, which can lead to the development of new materials with unique, enhanced properties [4, 5]. It is well known that nanomaterials exhibit characteristics different from those of macro-scale products [6-8] Recognized as the new frontier, nanotechnology generates new products with encouraging effects and significant impacts on both health and the environment [9, 10]. Nanoparticles (NPs) are characterized by a very high surface energy, which increases their contact capacity in relation to their volume [5, 11-13], explaining their distinct properties and heightened reactivity, along with unique quantum effects, configuring new properties and functionalities. As a result, nanomaterials have attracted attention and found applications in numerous fields. [7]. The unique properties of nanoparticles, including their high surface-to-volume ratio and antimicrobial effect, as well as controlled reactivity, offer several advantages compared to the properties of the same material at the macro-scale. [14].

The field of nanoparticles has attracted attention due to its variety of applications in various domains and its unique properties such as enhanced resistance, catalytic activity, optical properties, antimicrobial activity, and increased reactivity. The catalytic activity of nanoparticles is attributed to their size and structure, which provide a larger surface area-to-volume ratio, leading to greater adsorption capacity for chemicals and facilitating interaction with reactive molecules[5, 15].

The presence of nanomaterials in our lives manifests through beneficial contributions to health, with multiple applications in medicine, as well as in electronics, cosmetics, the food industry, automotive industry, and water delivery systems, resulting in significant outcomes such as increased efficiency and storage capacity[10]. However, it is crucial to continue research and find methods for obtaining and replicating products with improved characteristics[10, 16-18]. However, it is crucial to continue research and find methods for obtaining and replicating products with improved characteristics. Continuous research on nanomaterials is necessary to drive innovation in this field. Nanoparticles, with their enhanced properties, can offer solutions to current problems related to pollution and resource scarcity [19, 20]. Through their specific and personalized properties, nanoparticles lead to stronger, more durable materials, promising innovative materials for the future[9, 21].

II.2 Caracterization of Nanoparticles and their applications

The distinct properties of nanoparticles drive their utilization in the field of electronics as sensors, catalysts, or products with antibacterial effects. The plasmonic effect of metallic nanoparticles entails the phenomenon of plasmonic resonance, which involves the interaction between the incident electromagnetic field and the free electrons on the surface of nanoparticles. Plasmonic resonance, which can induce selective absorption and scattering depending on the size, shape, and composition of nanoparticles, can lead to properties such as fluorescence and phosphorescence [15, 22].

Nanoparticles have lower melting and boiling points compared to materials on a macro scale. This characteristic leads to easier melting and vaporization at lower temperature [23]. Nanomaterials have contributed to advancements in the production of high-performance composite materials, lightweight materials, and coatings with enhanced functionality [1, 6, 14, 24]. In materials science, nanomaterials are incorporated into mixtures with the purpose of enhancing the properties of composites [25, 26].

The antimicrobial activity of nanoparticles (NPs), particularly silver nanoparticles, has led to their utilization in various fields such as medicine, biology, biochemistry, electronics, and industry[27]. The antimicrobial activity of nanoparticles is a complex phenomenon with a mechanism that is still not fully understood regarding its action on microorganisms. However, it is crucial to mention that the effectiveness of nanoparticles against microorganisms depends on their shape, size, and the system in which they operate.[28-30].

II.3 Synthesis routes of Nanoparticles

The most commonly used methods for obtaining silver nanoparticles are physical, chemical, biological, or a combination of these methods. Each of the mentioned synthesis approaches offers unique advantages in terms of controlling the size, shape, and stability of the nanoparticles[9].

The chemical synthesis of silver nanoparticles (AgNPs) has specific characteristics depending on the reducing agents used, the physical conditions of the system, and the concentrations of the precursors. The synthesis of silver nanoparticles is relatively simple, and the duration of the chemical processes is relatively short and not costly. The most common method for synthesizing silver nanoparticles is liquid-phase synthesis, the stages of which can be controlled to achieve certain sizes and shapes of nanoparticles.

Chemical reduction of stable colloidal dispersions in water or organic solvents with organic or inorganic reducing agents is one of the commonly used methods, being also one of the methods with a relatively simple and economical process of obtaining.

Modification of the size and shape of nanoparticles can lead to significant properties of the nanoparticles. The study of the mechanism and antibacterial activity of silver ions and silver nanoparticles has shown that this property is strongly influenced by reaction conditions, the kinetics of interaction between metal ions and reducing agents, and the adsorption process of the stabilizing agent with metal nanoparticles[31]. This work will investigate the influence of reaction components on the physicochemical properties. The size and shape of nanoparticles are strictly dependent on the individual synthesis time, e.g., the nucleation process of nanoparticles, reaction temperature, and their growth time.

In this study, both the physical and microbiological aspects of silver nanoparticles resulting from various chemical reduction methods carried out at ambient temperature, 70°C, and high temperature in a controlled environment at constant pressure by the solvothermal method will be analyzed. The influence of reaction components on the formation of silver nanoparticles as well as their antibacterial efficiency will be examined. The silver nanoparticles

(AgNPs) obtained will be selected based on the technical analysis results and microbiological tests and will be used to test their antimicrobial activity on various substrates applied for protection and prolonging their resistance over time.

The unique properties of metal nanoparticles, especially silver nanoparticles characterized by strong antibacterial activities, have led to their diverse applications. The antimicrobial and antifungal effect is attributed to both the appearance and dimensions of silver nanoparticles as well as the release of silver ions, which have a toxic effect on microorganisms, including bacteria, fungi, and viruses[21, 32-35].

II.4 Biodegradability and state of various surfaces under conditions of excesive pollutions

Natural stone surfaces are vulnerable to biological deterioration caused by the colonization of microorganisms such as algae, mosses, and lichens. This is primarily due to the porous structure of the natural stone substrate, as well as human negligence[36-39].

The process of biodeterioration is defined according to standard EN15898 as an undesirable process in which the properties of a material are modified due to the activity of various microorganisms. The deterioration or alteration process of natural stones is a continuous cycle of disintegration and reconstruction, influenced by natural effects dependent on intrinsic and extrinsic factors. External factors such as temperature, pollution, and humidity are extrinsic, while factors like destruction, improper treatment of natural stone surfaces, and pollution all contribute to the degradation of materials [40, 41].

Rocks made of natural stone with high porosity, rough surfaces, and a carbonate structure, or those that cannot be finely processed resulting in polished surfaces, are most susceptible to biocolonization. Sedimentary rocks with a carbonate structure (such as limestone, travertine) or siliceous carbonate rocks (such as slate, quartzite, schist, certain limestone formations), as well as metamorphic rocks with high carbonate content (such as marble, onyx, aragonite, quartzite, etc.), are commonly encountered in construction areas, decorative arrangements, etc.The presence of microorganisms on affected surfaces leads to various modifications, including surface discoloration, cracking, exfoliation, disintegration, or increased water retention, even affecting their internal structure.

The use of nanoparticles in the conservation and protection of natural stones is one of the significant achievements of nanotechnology. Leveraging the properties of nanoparticles has allowed for the adaptation of these materials for both substrates and the functions they need to fulfill[42, 43]. The utilization of metallic nanoparticles and metal oxides, either individually or in combination, in the design of nanocomposites has led to the development of novel treatments for consolidation, hydrophobization, or biocidal purposes. Recent studies have assessed the presence of a mixture of consolidating hydrophobic agents and biocides in preventing biological growth on natural stone substrates[5, 44].

Nanoparticles represent an improved alternative with reduced toxicity levels. However, analyses and studies indicate that certain nanoparticles may have a higher level of toxicity. Nevertheless, studies regarding accumulations in flowing waters or within soil are not yet conclusive. For this reason, it is recommended to use them with caution and in the smallest possible quantities[45].

III. ORIGINAL PART

III.1 Chemical synthesis routes of silver nanoparticles

Chemical synthesis at room temperature involves the reduction of silver salt using reducing agents. The chemical synthesis process involves two reducing agents: trisodium citrate ($Na_3C_6H_5O_7$), which is a moderately reducing agent, and a strong reducing agent, namely sodium borohydride ($NaBH_4$).

III.2 Reduction synthesis at room temperature

Chemical synthesis with reducing agents at room temperature has led, through varying the volume of the strong reducing agent ($NaBH_4$), to obtaining several types of silver nanoparticle (AgNPs) solutions predominantly with a blue color (Figura 1)[46]. The variation in the volume of the strong reducing agent ($NaBH_4$) aims to establish an optimal ratio between the reducing agents for obtaining AgNPs that can subsequently be applied as antimicrobial coatings.

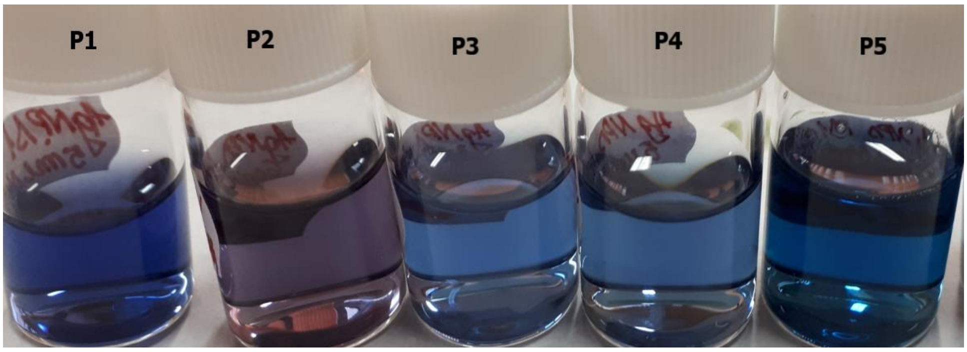


Figura 1 Various suspensions of AgNPs obtained via chemical method at room temperature selected for testing P1 and P4 samples with a molar concentration of 0,06 M; P3 and P5 samples with molar concentration 0,1 M; P2 sample with molar concentration of 0,5M

III.3 Synthesis conducted at room temperature using the Dong method

The Dong synthesis aimed to obtain triangular-shaped nanoparticles, knowing that these are characterized by increased antibacterial efficacy due to their high capacity for penetrating bacterial cell walls.[31, 47].

The obtained sample exhibits a reddish-brown coloration, indicating the presence of silver nanoparticles with estimated dimensions around 20-40 nm.[48]. The pH of the obtained solution is 8, and the concentration achieved is 300 ppm.

III.4 Synthesis of AgNPs at temperature

III.4.1 Reduction synthesis conducted at 70°C

The synthesis conducted at temperature differs from the first two syntheses both in terms of the quantities of precursors used and the reaction conditions. By varying the reducing agent $NaBH_4$ throughout the synthesis reaction, the aim was to obtain AgNPs with antimicrobial activity.

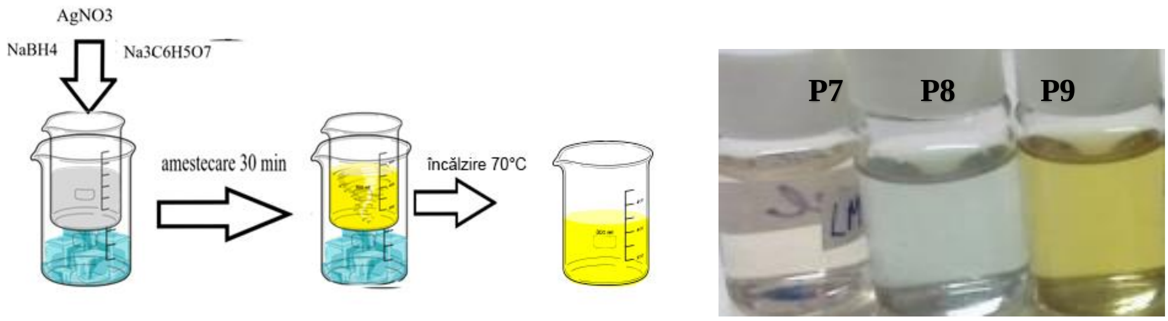


Figura 2 Obtained scheme of AgNPs supensions with at temperature

III.4.2 Solvothermal synthesis of AgNPs

The solvothermal synthesis is a precise method for obtaining nanoparticles, where the temperature factor allows precise control over their size, shapes, and distribution. The method uses polyethylene glycol as the reducing agent and polyvinylpyrrolidone as the protective agent, the same products as in the previous syntheses (Figura 3). Unlike the previous syntheses, the solvothermal process is considered a heterogeneous reaction in the presence of solvents and under the action of pressure and temperature, where, due to special conditions, the reactants increase their solubility, thus increasing the nucleation rate.[49, 50].

The solvothermal method has several advantages over classical chemical reduction [51, 52]. [53, 54]. The reaction using the solvothermal method was carried out at various temperatures to observe if there are any changes in the appearance, shape, and dimensions of the Ag nanoparticles and to determine if there is a specific temperature limit at which silver nanoparticles can undergo significant modifications.

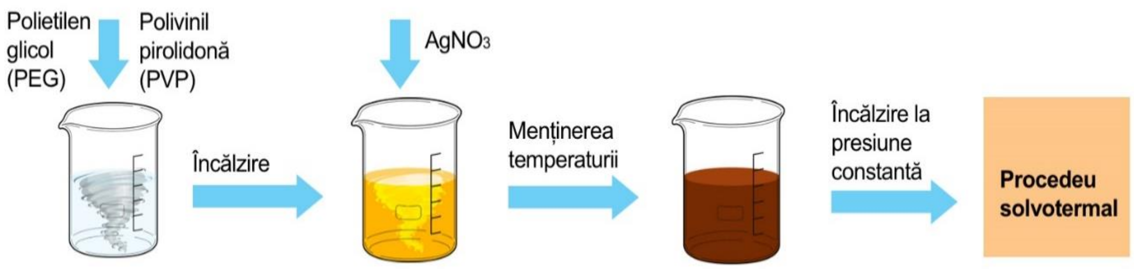


Figura 3 Obtained scheme for AgNPs suspensions through solvothermal method

The samples resulting from the solvothermal method at different temperatures and times exhibit a varied coloration ranging from yellowish-red to dark brown (Figura 4), characterized by higher viscosity compared to the two methods used previously[52, 53, 55], the pH of the solutions is around 9, and the concentration is 1000 ppm. The samples obtained at high temperature, in both cases, were coded from P10 to P14.

Figura 4 Aspect of AgNPs suspensions obtained by solvothermal method

III.5 Antimicrobial analysis

III.5.1 Antimicrobial charcaterization of AgNPs obtained through chemical synthesis

The UV-Vis analysis for the samples obtained through chemical synthesis at room temperature (P1-P5) (Figura 5) confirmed the presence of silver nanoparticles, with each synthesis exhibiting its own characteristics depending on how the reaction process was conducted. The UV-VIS results for the synthesis carried out at room temperature (method I) confirm the presence of silver nanoparticles. In this case, each sample is characterized by two or three maxima located around the values of 330-340 nm, 400-420 nm, and 600-800 nm.

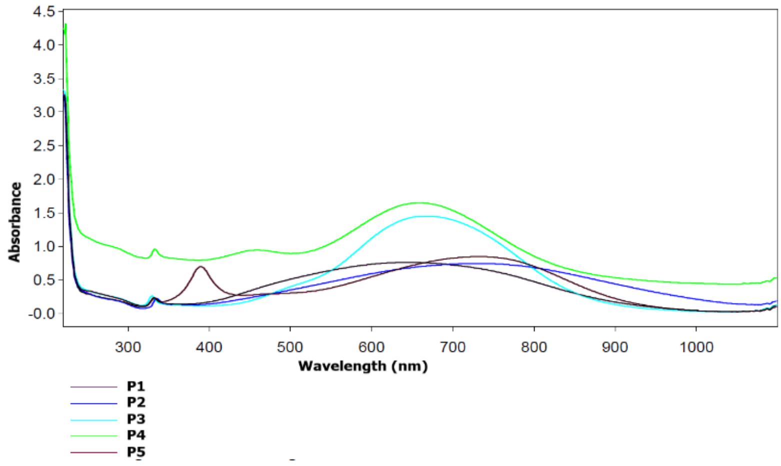


Figura 5 UV-VIS for AgNPs susensions obtained at room temperature

III.5.1.1 Dynamic Light Scattering analyses

The comparative analysis of the results obtained from the DLS analysis for the synthesis carried out at room temperature provides information regarding the stability of nanoparticle solutions as well as their dimensions. For samples P1-P5, whose synthesis is identical except for the volume of the strong reducing agent, sodium borohydride ($NaBH_4$), it is observed that the zeta potential (ζ) of the formed nanoparticles is greater than -30 mV, confirming their stability.

In the case of the Dong-type synthesis, sample P6 exhibits a relatively uniform distribution with nanoparticle sizes around 30-40 nm. The moderately reducing agent (trisodium citrate) leads to the formation of a limited number of nanoparticles that can aggregate to form clusters. In this case, trisodium citrate acts as both a reducing agent and a protective agent [18, 56-60].

III.5.1.2 TEM analyses

The TEM analysis of samples obtained through room temperature synthesis, namely P1-P5, conducted for two samples, P3 and P1, leads to the conclusion that the samples exhibit different shapes, characterized by anisotropy (Figura 6a,b) with the formation of individual crystalline structures grouped in parallel in a multiply twinned manner (Figura 6 a)[61]. The irregular shapes were confirmed by UV-VIS results, as indicated by the positioning of the peak maxima towards values greater than 600 nm and the broad curve shapes corresponding to the two analyzed samples.

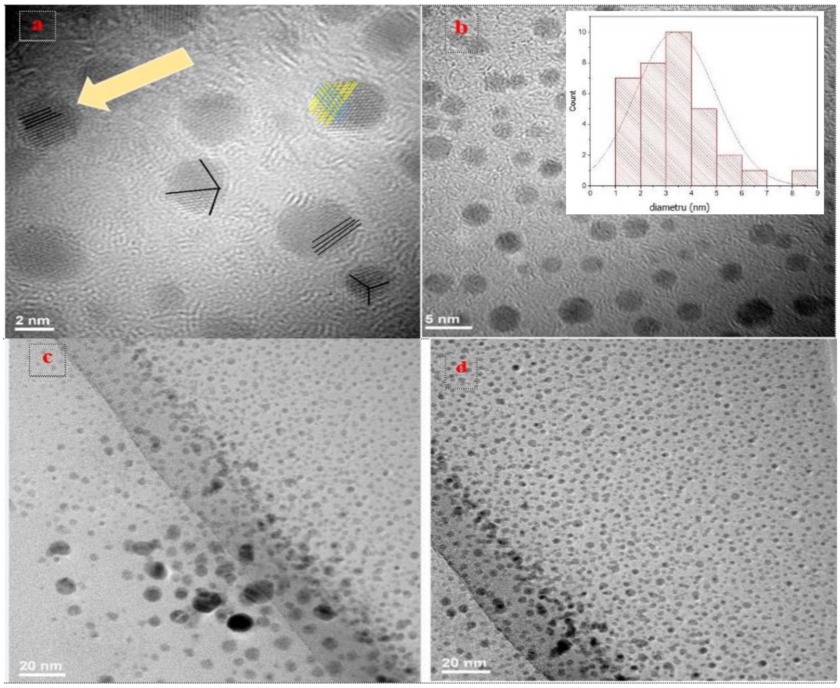


Figura 6 Imagini TEM pentru proba P3 a)imagine TEM reprezentând nanoparticule cu formă sferice b)imagine TEM forma de nanoprticule formă sferică, în partea dreapta histograma reprezentând distribuția nanoparticulelor c-d) nanoparticule cu dimensiuni relativ mici, distribuite uniform și cu usoară tendința de aglomerare

III.6 Results of chemical reduction at room temperature

The UV-Vis analysis obtained for the AgNPs synthesis carried out at a temperature of 70°C confirms the presence of nanoparticles (Figura 7) for the indicated samples P7 and P9, with varying content of the strong reducing agent sodium borohydride ($NaBH_4$).

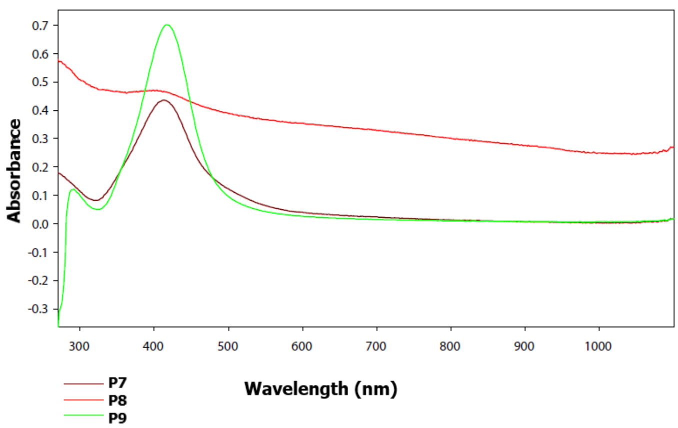


Figura 7 UV-VIS for AgNPs suspensions obtained at temperature 70°C

The presence of nanoparticles formed at temperatures between 200°C and 260°C is indicated by the graph (*Figura 8*) and confirms that their sizes are relatively small, as indicated by the UV-Vis values (400 nm). The characteristic peaks corresponding to these values, for samples P10, P13, and P14, indicate the presence of multiple nanoparticles with sizes that are relatively close. Essentially, temperature and pressure factors can significantly influence the likelihood of their formation, and the shape and dimensions of the synthesized nanoparticles are likely influenced by these factors [49].

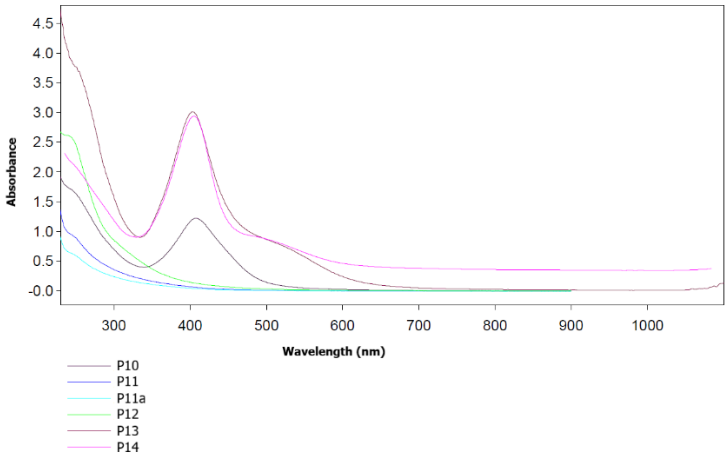


Figura 8 UV-VIS AgNPs samples obtained by solvotermal method

III.6.1 DLS analyses for samples synthetized at temperature

The DLS (Dynamic Light Scattering) images obtained from the analysis of samples synthesized via the solvothermal method indicate a polydispersity of the obtained nanoparticles and also the formation of clusters with sizes larger than 50 nm.

III.6.2 TEM analyses of samples synthetized at temperature

The samples synthesized via the solvothermal method, the presence of nanoparticles with large sizes is indicated, as well as a distribution of small nanoparticle sizes that tend to coalesce or aggregate.

TEM analysis of sample P10 confirms the presence of spherical-shaped nanoparticles (Figura 9) and a relatively homogeneous distribution, with a tendency for AgNPs to agglomerate.

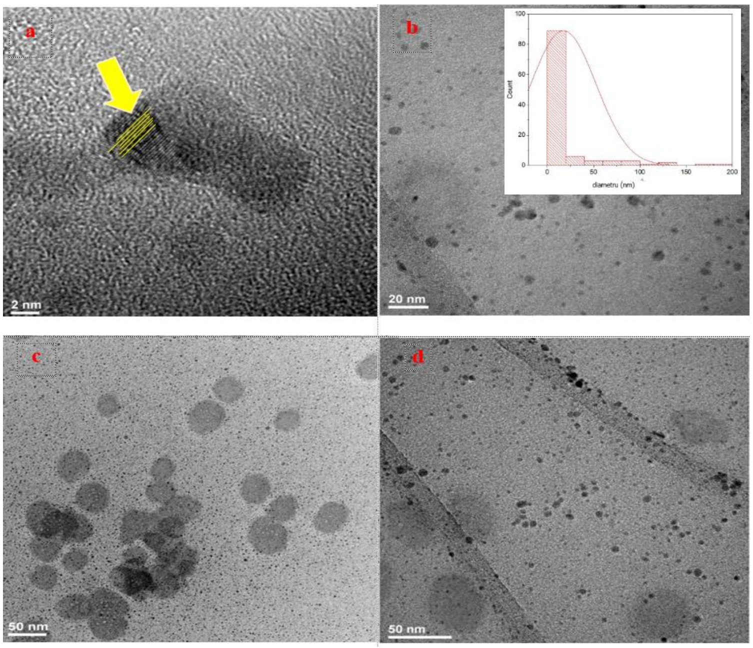


Figura 9 TEM images for sample P10 obtianed via solvothermal synthesis a) TEM images confirm the formation of multiply cristaline twinned plans b,c,d) TEM images at different resolutions showing AgNPs with a tendency to agglomerate; histogram of te size distribution of the formed nanoparticles

TEM analysis of sample P13, conducted at the highest temperature compared to the other samples, indicates the formation of spherical-shaped nanoparticles with relatively medium sizes but with a pronounced tendency for agglomeration and coalescence, as indicated by the data (*Figura 10c).*

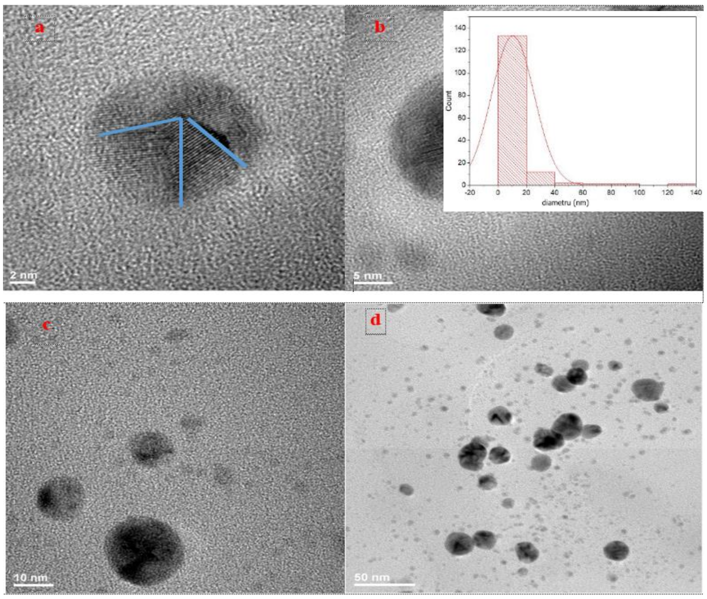


Figura 10 TEM images for P13 sample obtained via solvothermal synthesis at 260°C a) TEM images confirmed the formation of multiply cristaline twinned planes b,c,d) TEM images at different resolutions of AgNPs with spherical morphology; histogram of the size distribution of the formed nanoaprticles

TEM analysis of sample P14 obtained at a temperature of 220°C confirms the presence of predominantly spherical-shaped nanoparticles (Figura 11) as also supported by the UV-Vis data with the predominant peak formed around the value of 400 nm. This indicates the presence of silver nanoparticles of spherical shape, corroborated with the results obtained from the DLS analysis, which indicate small sizes of the formed nanoparticles in sample P14.

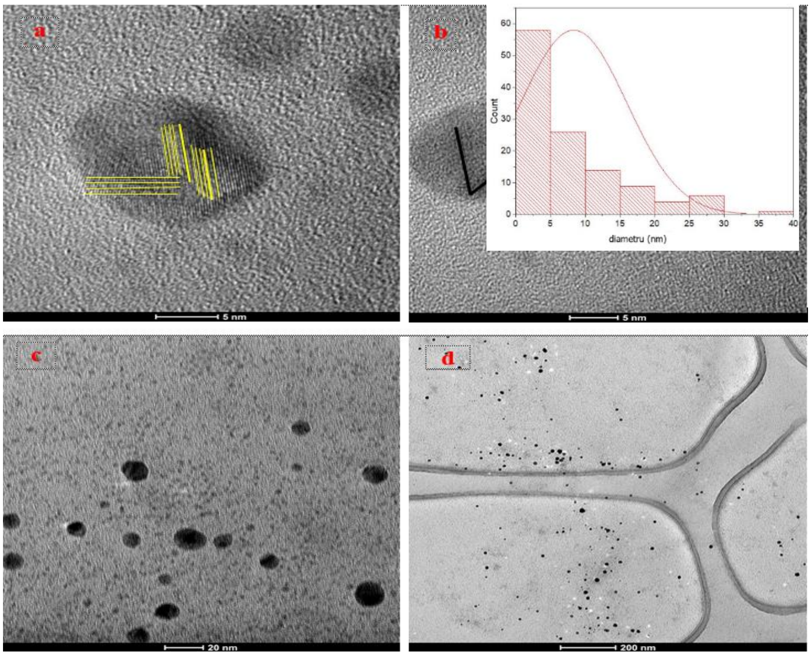


Figura 11 TEM images for sample P14 obtained via solvothermal synthesis a si b) TEM images confirm confirmă formation of multiply twinned plannes; c,d) TEM images at different resolution with AgNPs with spherical morphology; histogram of the size distribution with agglomeration tendency

III.6.3 Antimicrobial activity

The antimicrobial activity was evaluated by comparing the inhibition zone (average zone diameter of inhibition) against various strains[35]. All samples obtained at room temperature (Figura *12*) exhibit strong antimicrobial activity against the *S. aureus* strain. The shape of AgNPs obtained through this method and their specific sizes ranging from 1-10 nm determine better antimicrobial activity and efficacy against *S. aureus* strains. For samples of AgNPs obtained at high temperature, a weak antimicrobial activity is observed, manifested by the development of a reduced zone of growth inhibition (ZOI). Samples P10 and P13 obtained at temperatures of 200°C and 260°C, respectively, which present spherical-shaped AgNPs and predominantly nanoparticle sizes around 20 nm, exhibit very good antibacterial efficiency against the *S. aureus* strain.

Comparatively, with samples obtained at room temperature, it was observed that samples synthesized via the solvothermal method (P10 and P13) also demonstrated better antimicrobial activity.

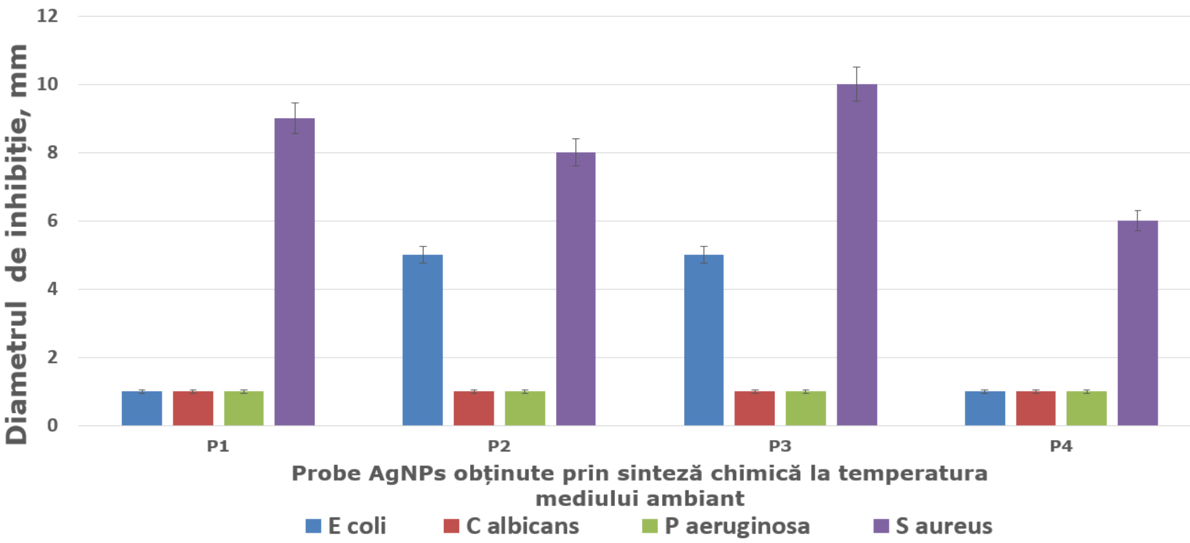


Figura 12 The diameter of the growth inhibition zone of microorganisms after cultivation on solid media in the presence of NPs obtained by the chemical method at room temperature

The antibiofilm activity confirms that samples obtained through the solvothermal method have increased antibacterial activity compared to samples obtained at room temperature for both Gram-positive and Gram-negative bacteria (*Figura 13*). The anisotropic shape and relatively small sizes of nanoparticles obtained at room temperature, compared to those obtained through the solvothermal method, contribute to the enhancement of their antimicrobial activity. It is worth mentioning that samples P10-P13 obtained via solvothermal route recorded nanoparticle sizes with diameters exceeding 50 nm, and it is possible that at larger sizes of silver nanoparticles, the mechanism of attack on microorganisms may be relatively efficient[19].

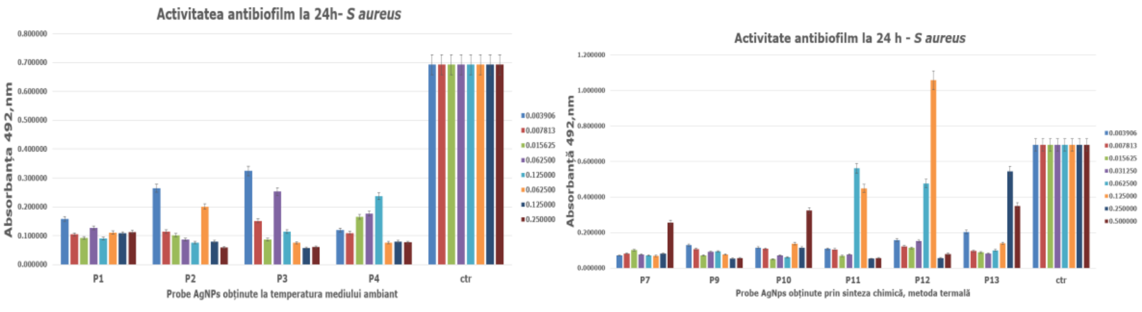
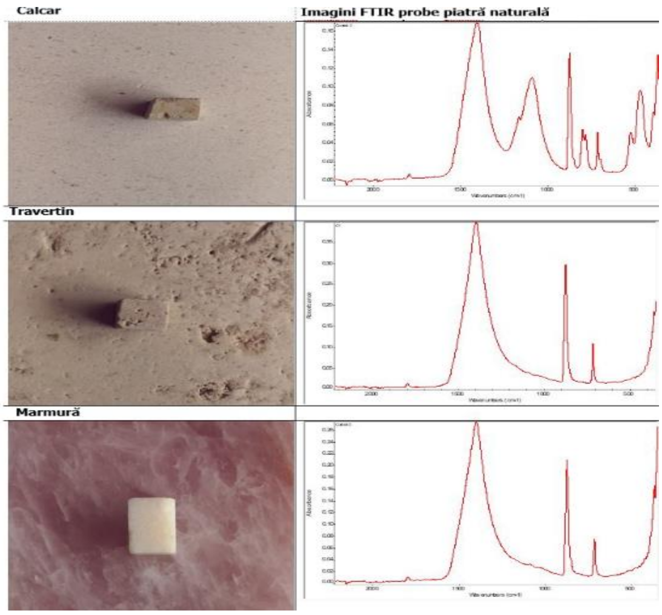


Figura 13 The graphical representation of absorbance values at 492 nm illustrating the antibiofilm activity against *S aureus* cultured for 24 h in liquid medium in the presence of different concentration of AgNPs obtained through synthesis at room temperature and solvotheral synthesis.

IV. APPLICATION OF SYNTHETIZED OF SILVER NANOPARTICLES ON VARIOUS NATURAL STONE SUBSTRATES FOR ANTIMICROBIAL, ANTIFUNGAL PROTECTION

For the testing of the silver nanoparticles described in the first experimental part, various types of natural stone were used as substrates for protection tests, including marble, travertine, and limestone (Tabel 1). The testing utilized those types of natural stone commonly used in construction and finishing, predominantly sedimentary and metamorphic limestone rocks [43].

Tabel 1 Types of substrates used for impregnation method



The natural stone samples were impregnated with coupling agents using silanization products with reactive thiol (-SH) and amino (-NH2) groups. The impregnation process described earlier involves treating the surfaces with coupling agents that bind, in our case, silver nanoparticles to the surface to be treated. The free silanol groups can form covalent bonds with the silver nanoparticles and can be arranged in a single layer or multilayer configuration (Figura *14*).

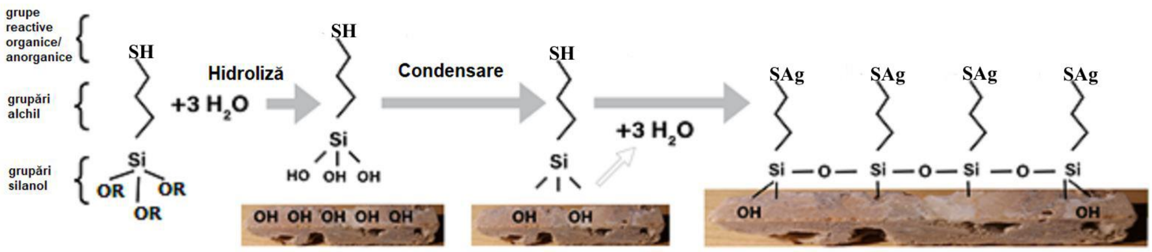


Figura 14 General scheme illustrated the adsorbtion of AgNPs on treates substrates

IV.1 RESULTS

IV.1.1 X-ray diffraction analysis of natural stone substrates

The X-ray diffraction analysis (for each natural stone sample) yielded the results presented in (Figura *15*). The crystalline structure of the three types of natural stone samples indicates the presence of calcite, which is specific to the composition of certain sedimentary, carbonate rocks.

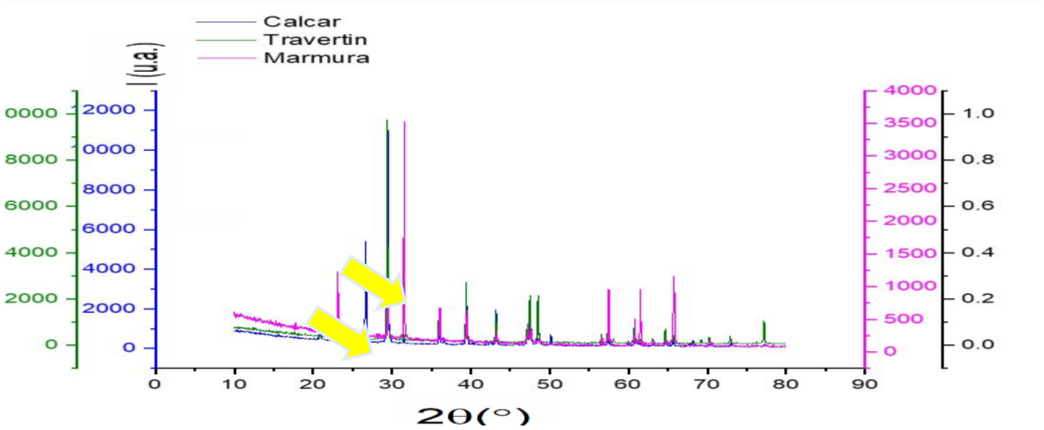


Figura 15 X-ray diffraction analyze for limestone, travertine, marble samples

IV.1.2 Results of treatment on various natural stone surfaces treated with 3APTES coupling agent

In the case of treating the limestone surface with different concentrations of the coupling agent, the presence of vibration frequency around the value of 1090-1020 cm^{-1} indicates the presence of primary amino group ($-NH_2$).

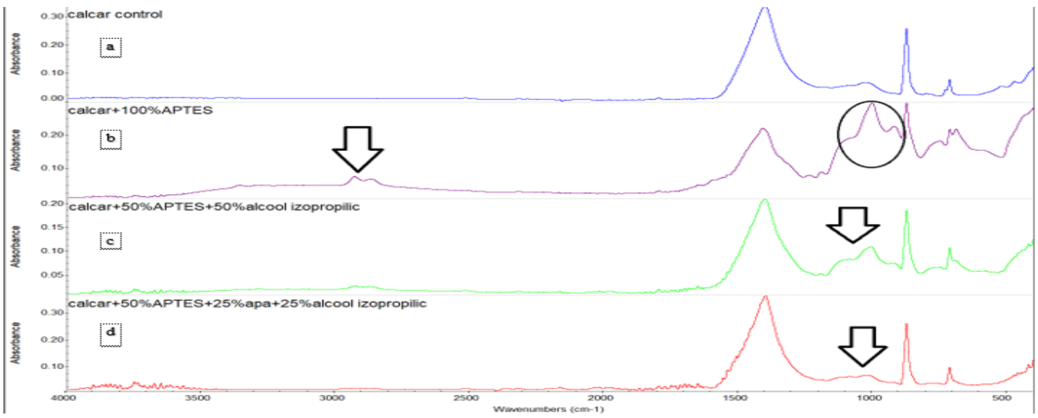


Figura 16 FTIR images processed on limestone samples treated with 3APTES and mixing

IV.1.3 Results of treatment on various natural stone surfaces treated with 3MPTMS coupling agent and a mixture of 3MPTMS and izopropyl alcohol.

In the treatment of travertine substrates with a mixture of 3MPTMS and isopropyl alcohol, respectively a mixture with isopropyl alcohol and water, the FTIR images do not indicate any difference. This confirms that on a porous layer, the adsorption of the mixtures is less influenced by the presence of water in this proportion (*Figura 17*).

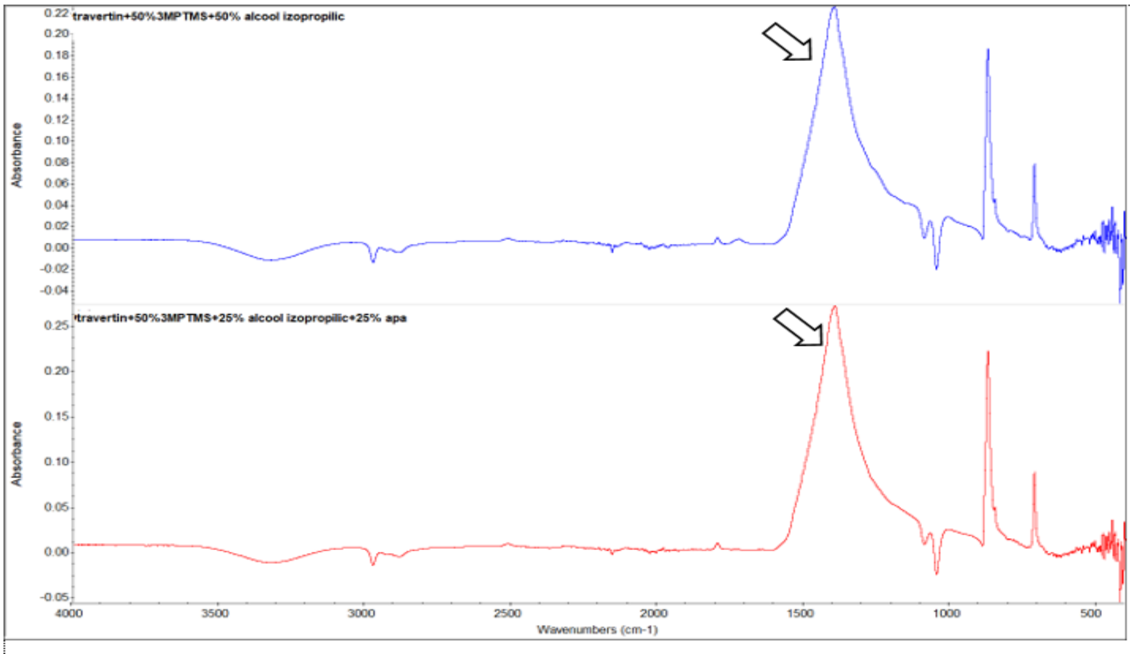


Figura 17 FTIR comparative images of the treated travertine with mixing of 3MPTMS, izopropylic alchohol and water

IV.1.4 FTIR map imaging for surfaces treated with 100% coupling agents or mixtures

The FTIR map images for substrates treated with coupling agents like 3MPTMS and Coat OSil T cure are analyzed comparatively based on the types of substrates they are applied on. In the case of treatment with 100% 3MPTMS and a mixture of 50% 3MPTMS with 50% isopropyl alcohol (IPA), it is observed that regardless of the substrate, the presence of thiol-type groups is confirmed (710-685 cm^{-1} sau 705-570 cm^{-1}).

In the case of travertine treated with 100% 3MPTMS and a 50% 3MPTMS and 50% IPA mixture (Figura *18*), variations in surface adsorption are observed in both cases. Spectral analysis (Figura *18*) on the travertine substrate treated with 100% 3MPTMS confirms the presence of C-S type bonds. The FTIR map image for both silanizations confirms that the interaction between the substrate and the coupling agent is relatively moderate to diminished.

This is primarily due to the porous structure of the substrate, characterized by open pores through which the coupling agent is adsorbed relatively strongly on its surface.

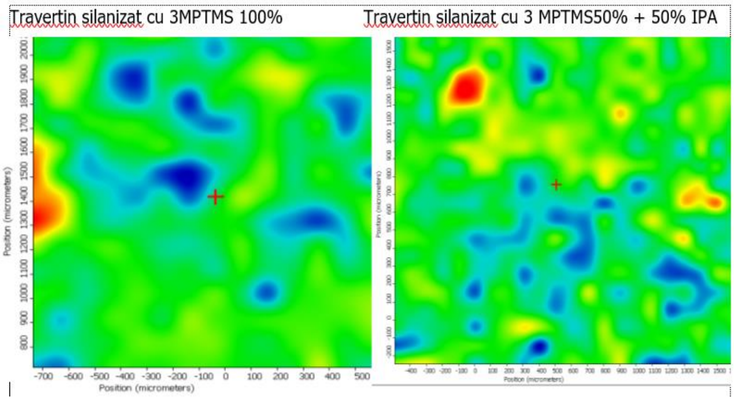


Figura 18 FTIR map for travertine substrate treated with a) 100% 3MPTMS; b) 50% 3MPTMS+50% IPA

Analysing the limestone or marble substrates, a smaller variation in the adsorption of the 100% 3MPTMS coupling agent is observed, but relatively better adsorption is observed in limestone when mixed with IPA. This is due to their homogeneous structure compared to the structure of travertine (*Figura 19*). In the case of the marble substrate, although it has a compact crystalline structure, the FTIR map images (Figura *20*) show weak interaction of the silanization agent with the substrate, a case confirmed by the FTIR image results. It is possible that the physical properties of the 3MPTMS silanization agent do not match this type of substrate, thus reducing its adhesion.

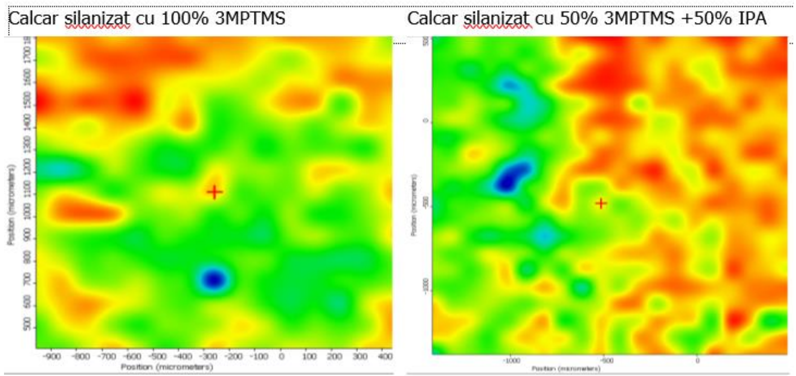


Figura 19 FTIR map on tretated limestone with a) 100% 3MPTMS; b) 50% 3MPTMS+50% IPA

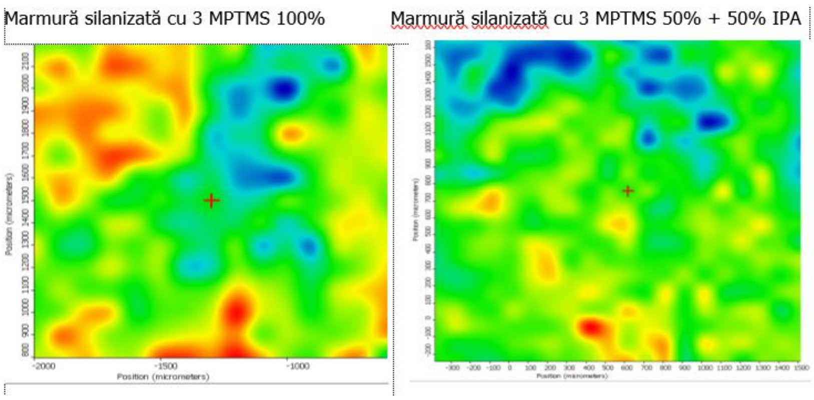


Figura 20 FTIR map of treated marble with a) 100% 3MPTMS; b) 50% 3MPTMS+50% IPA

In the case of limestone, both silanization with 100% Coat O sil coupling agent and in a mixture (50% Coat O sil + 50% IPA) show relatively better interaction with the coupling agent compared to travertine (*Figura 22*). The FTIR map images are relatively homogeneous in the case of the limestone substrate (Figura *22*) compare with the travertine substrate (Figura *21*).

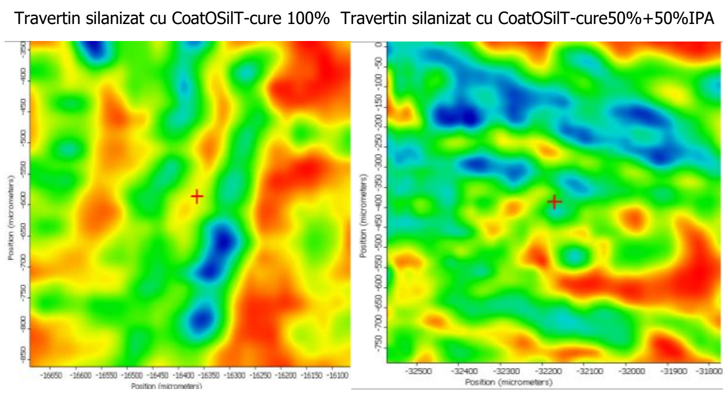


Figura 21 FTIR map of treated travertine with a) 100% Coat O Sil; b) 50% Coat O Sil+50% IPA

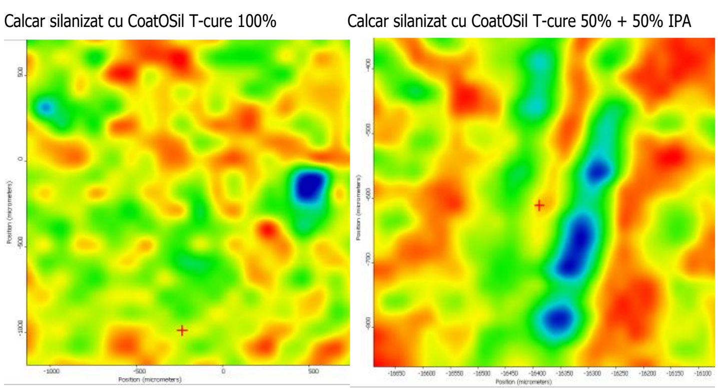


Figura 22 FTIR map of treated limestone with a) 100% CoatOSil; b) 50% Coat O Sil+50% IPA

IV.2 Surface of characterization of treated surface with coupling agents by Scanning Electron Microscopy (SEM)

The comparative SEM images provide clues about the silanization process of the surfaces regardless of their structure. The SEM images (*Figura 23*) indicate the formation of a film on the surface of the treated substrates, forming a compact layer. Compared to the SEM images (*Figura 24*,*Figura 25*) representing the coupling agents with thiol groups, which confirm the silanization of the surfaces by partially closing the microcracks and pores, thus allowing the substrate to "breathe".

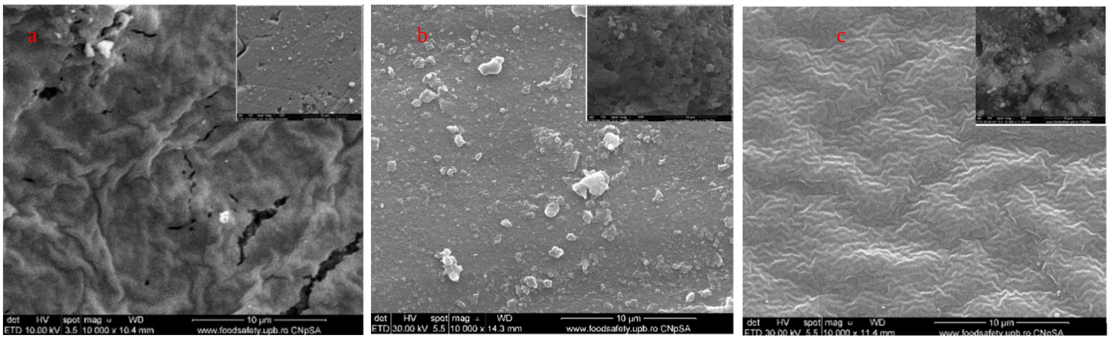


Figura 23 SEM images depicted various substrates treated with 3 aminopropyltriethoxysilane (3APTES) a) travertine; b)limestone; c) marble

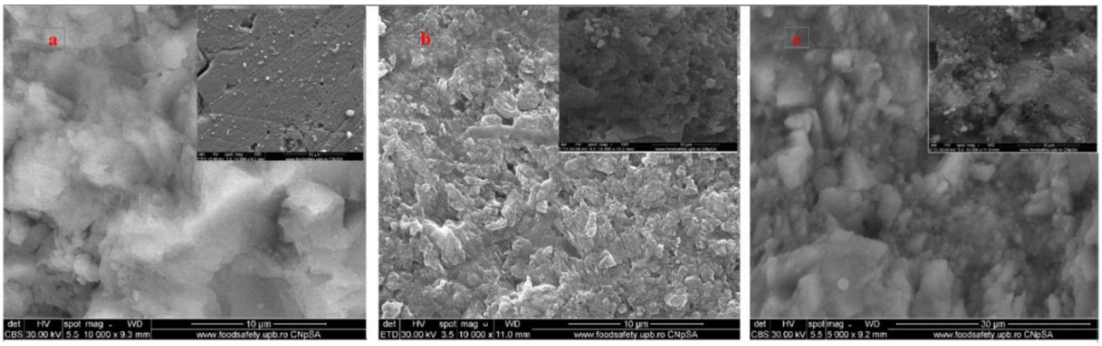


Figura 24 SEM images depicted various substrates treated with 3 MPTMS a) travertine; b)limestone; c) marble

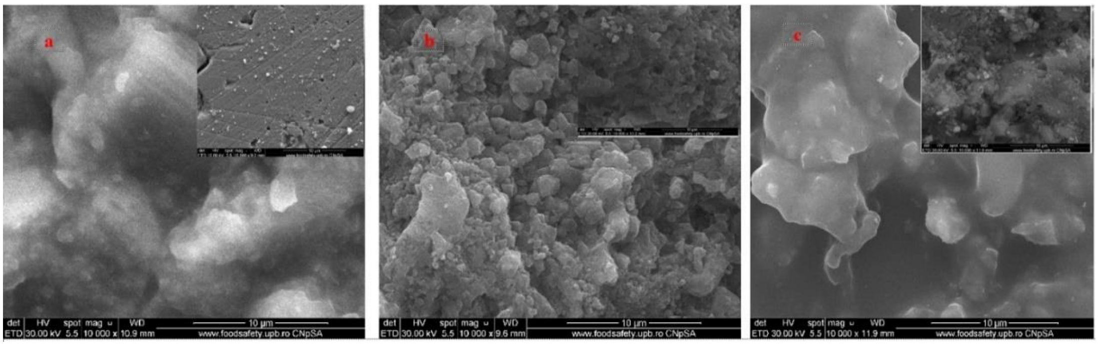
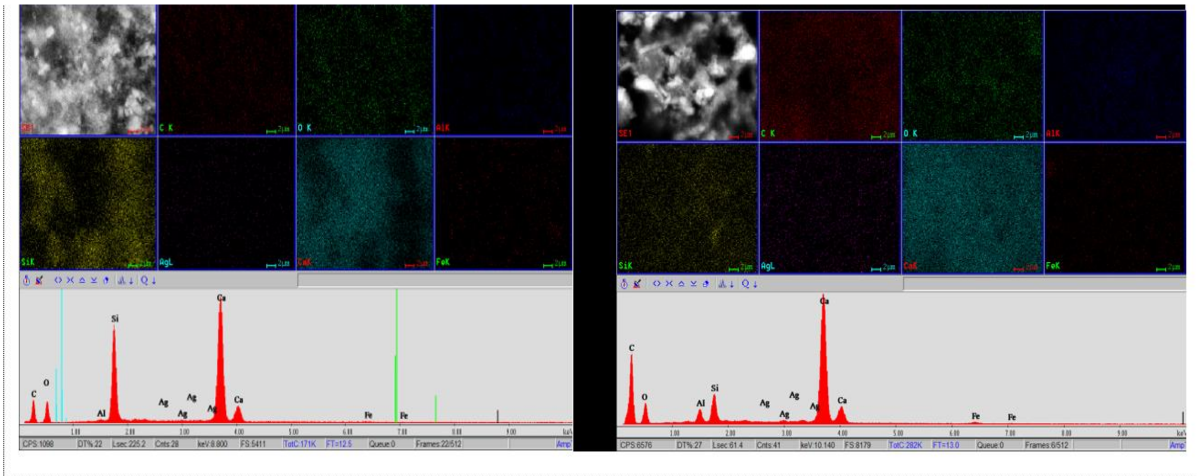


Figura 25 SEM images depicted various substrated treated with Coat O Sil T-cure a) travertine; b)limestone; c) marble

IV.3 Treatment of natural stone surface with silver nanoparticles (AgNPs)

IV.3.1 SEM treated surface characterized with AgNPs

The presence of AgNPs was revealed by BSED analyze on natural substrates treated with coupling agents type 3 MPTMS (Figura 26).



calcar +3 MPTMS + AgNps 1000 ppm travertin +3 MPTMS + AgNPs 1000ppm

Figura 26 Secondary electron (BSED) images and surface intensity maps of characteristic X-ray radiation of the main chemical elements detected by EDS analysis .

IV.3.2 Surface characterization of substrates treated with AgNPs through UV-Vis analysis

The UV-Vis analysis of the AgNPs solutions (Figura 27) used for treating the surfaces of natural stone to highlight whether AgNPs have been adsorbed on the surface involves adding samples of the natural stone into the respective solution. Each sample is pre-impregnated with a coupling agent, such as 3APTES, 3MPTMS, or CoatOSil.

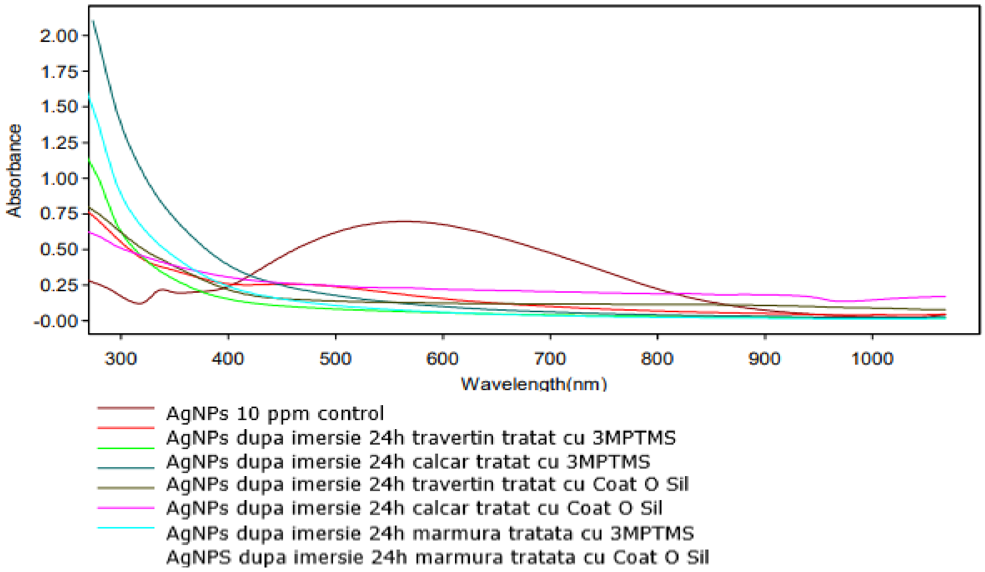


Figura 27 UV-VIS graphic for AgNPs suspensions with 10 ppm after immersing stone samples treated with coupling agents

IV.4 Antimicrobial surfaces treated with AgNps

IV.4.1 Antimicrobial activity of AgNps on natural stone treated with 100 % 3MPTMS coupling agent

In the case of treating natural stone substrates with the coupling agent 3MPTMS, the effectiveness of AgNPs against a Bacillus subtilis strain is analyzed for variants where the concentrations of AgNPs are 10 ppm and 1000 ppm (*Figura 28*). The *B. subtilis* species was chosen for testing because it is a ubiquitous, spore-forming, prokaryotic microorganism with high resistance to environmental factors, which colonizes natural stone surfaces and can contribute to their biodeterioration.

For the limestone substrate, it is observed that treatment with 3MPTMS and AgNPs, regardless of their concentration, confirms a low adhesion of *B. subtilis* cells of about 3-4 logarithmic units compared to the control sample.

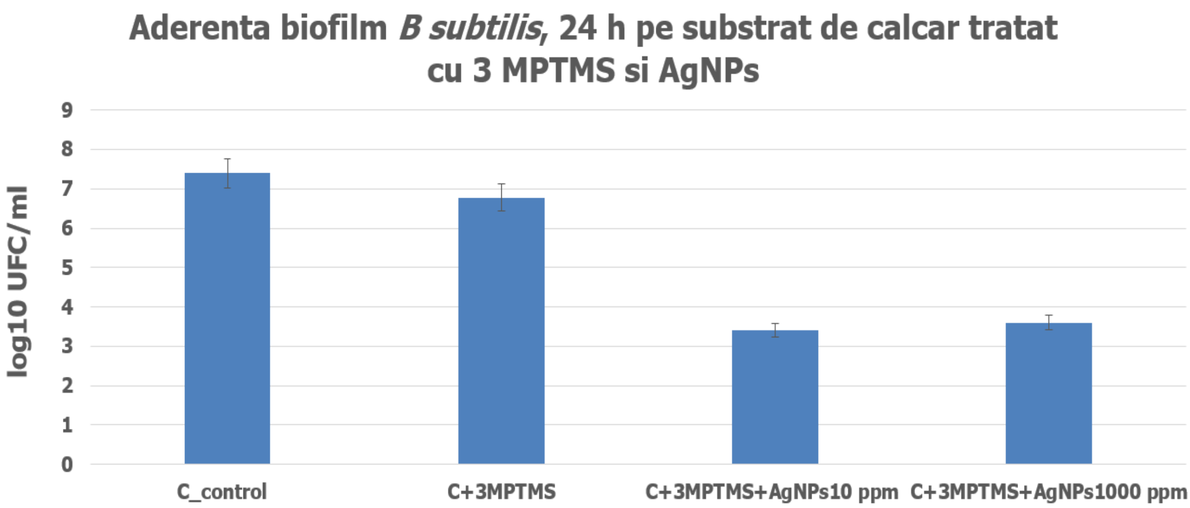


Figura 28 Graph representing the biofilm adherence of *B subtilis* on untreated limestone (C) control (control) și and treatment with coupling agents 3MPTMS and AgNPs at 10 and 1000 ppm

Regarding the treatment of the marble substrate with the coupling agent 3MPTMS and AgNPs at 10 ppm and 1000 ppm (Figura 29), a decrease in the development of *B. subtilis* biofilm by 2.8 logarithmic units was recorded for the sample treated with AgNPs at 1000 ppm compared to the control sample. In the case of treating the substrate with AgNPs at 10 ppm, the adsorption of the coupling agent on the substrate surface leads to a decrease in the fixation effect of nanoparticles on the surface, resulting in low efficiency values for these nanoparticles**.**

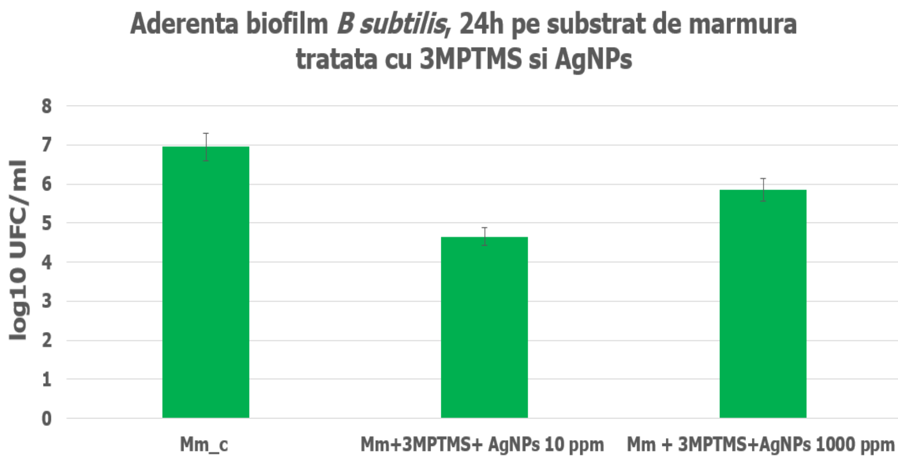


Figura 29 Graph representing the biofilm adherence of *B subtilis* on treated marble substarte with 3MPTMS100%

IV.4.2 Antimicrobial activity of AgNps on natural stone surfaces treated with 100% Coat O Sil T–cure coupling agent

In the case of treating the limestone and travertine natural stone substrates with the coupling agent Coat O Sil and AgNPs at 10 ppm and 1000 ppm (Figura 30) it was observed that for the limestone substrate, the results are similar for treatment with AgNPs at 10 ppm and 1000 ppm. There is a decrease in the development of *B. subtilis* biofilm by approximately 4.5 logarithmic units when treated with AgNPs at 1000 ppm and 4 logarithmic units when treated with AgNPs at 10 ppm (Figura 30). It is possible that the size and shape of the nanoparticles at 1000 ppm are more effective on the limestone substrate compared to travertine (**Error! Reference source not found.**).

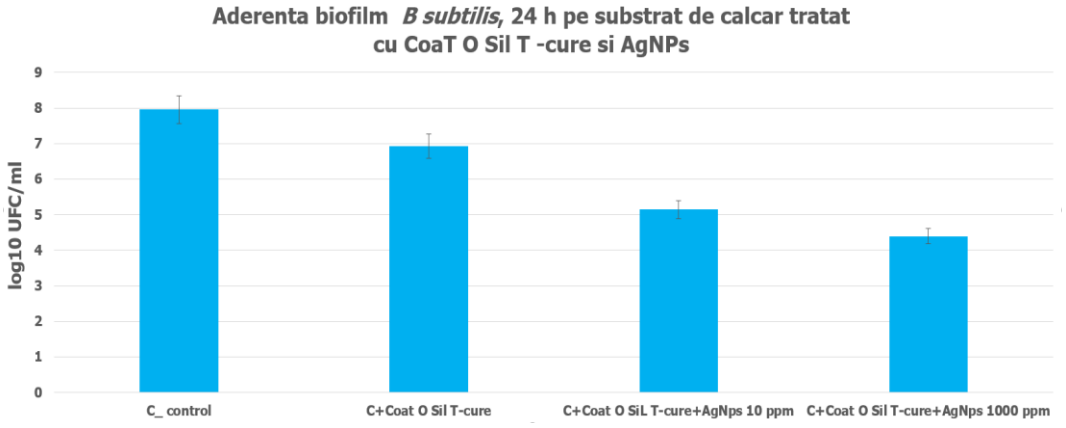


Figura 30 Graph representing the biofilm adherence of *B subtilis* on treated limestone with 100% Coat O Sil and AgNPs with various concentrations 10 and 1000 ppm

IV.4.3 Antimicrobial activity o AgNPs on stone surfaces treated with a mixture of 50% Coat O Sil T-cure and 50% izopropyl alcohol (IPA)

The treatment performed with a mixture of Coat O Sil 50% and isopropyl alcohol (IPA) 50% (Figura 31) on the limestone substrate resulted in observations regarding the influence of the presence of isopropyl alcohol in the mixture. Compared to the control sample of limestone and the one treated with 100% Coat O Sil coupling agent (Figura 31), the presence of isopropyl alcohol causes the biofilm adherence to decrease by 3 logarithmic units compared to the 2 units recorded by the sample treated with 100% Coat O Sil.

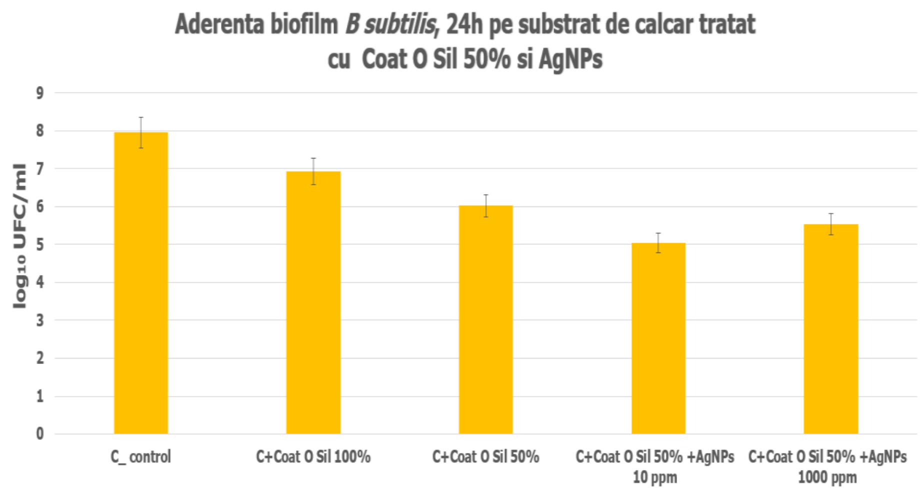


Figura 31 Graph illustration the adhesion capacity and biofilm formation of *B subtilis* on treated limestone substrate with 50% Coat O Sil and AgNPs concentrations of 10 și 1000 ppm

The treatment performed with a mixture of Coat O Sil 50% and isopropyl alcohol (IPA) 50% (Figura *32*) on the travertine substrate resulted in different outcomes compared to those obtained with the treatment using 100% Coat O Sil. Thus, the presence of isopropyl alcohol causes the biofilm adherence to decrease by 3 logarithmic units in the sample treated with AgNps 10 ppm and by 3.5 logarithmic units in the sample treated with AgNPs 1000 ppm compared to the sample treated with 100% Coat O Sil.

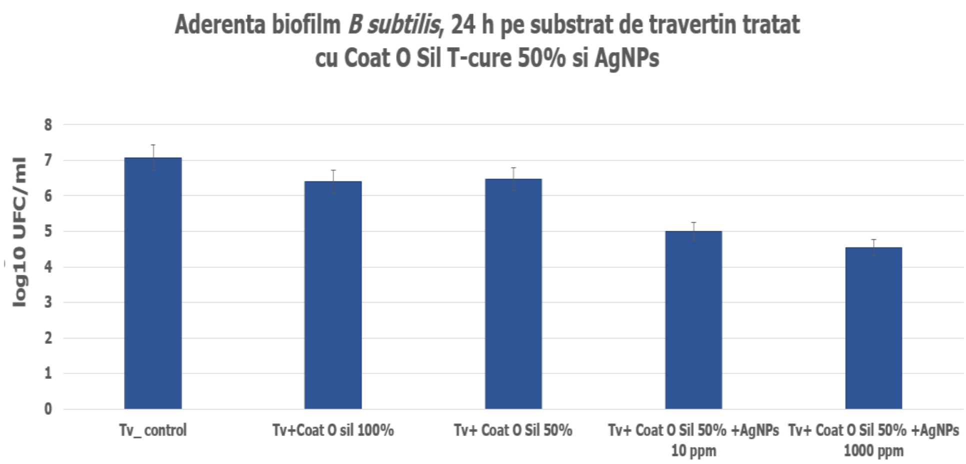


Figura 32 Graph illustration the adhesion capacity and biofilm formation of *B subtilis* on travertine substrate treated with 50% Coat O Sil and AgNPs concnetrations de 10 și 1000 ppm

IV.4.4 Antimicrobial activity of AgNPs on the natural stone surfaces treated with 3APTES as compared with 3APTES and methyl iodide (CH_3I)

The treatment of natural stone substrates with a mixture consisting of 3APTES (coupling agent) and methyl iodide (CH_3I) aims to treat them with quaternary ammonium compounds that act as biocidal products [62, 63]. The graph (*Figura 33*) confirms in the first stage that the mixture of 3APTES and methyl iodide is effective, recording a decrease in biofilm adherence values by 2 logarithmic units compared to the control sample.

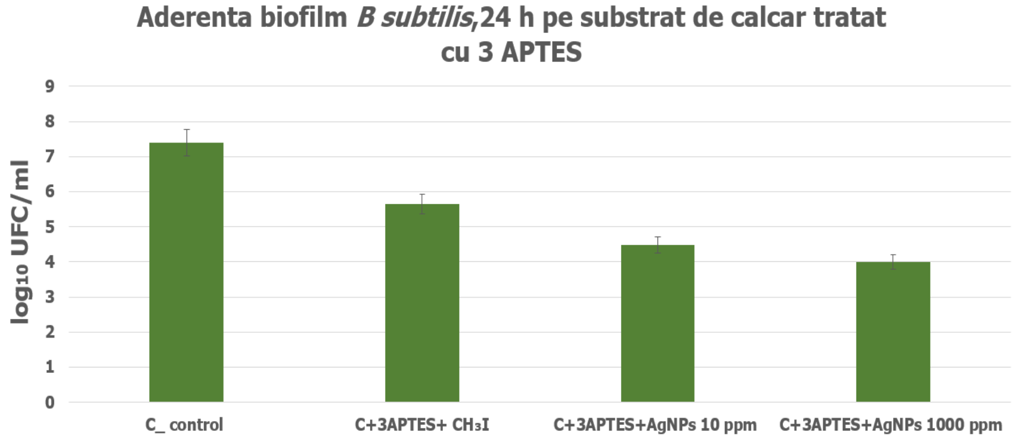


Figura 33 Comparative graph representing th adhesion and biofilm formation of *B subtilis* on limestone substrate treated with 3APTES and CH_3I respectively, 3APTES and AgNPs

IV.4.5 Antibiofilm activity of AgNPs on stone surfaces treated with coupling agents and exposed outdoor for a period of 3 month

The treatment of natural stone substrates with coupling agents 3MPTMS and Coat O Sil, followed by the application of AgNPs and exposure to outdoor conditions, aimed to determine the resistance of AgNPs under exterior conditions. Thus, the materials treated with AgNPs and previously evaluated coupling agents were kept outdoors for 3 months, after which the antibiofilm effect was re-evaluated using Bacillus subtilis as a model strain. The results obtained from the assessment of *B subtilis* biofilm formation on the samples exposed outdoors, compared to the data from the laboratory-obtained samples, are presented in graphs.

Analysis of the travertine substrate treated with 3MPTMS and AgNPs exposed outdoors from the graph (Figura 34) indicates that the data are similar to the samples obtained and analyzed earlier in the laboratory immediately after treatment. Both AgNPs 10 ppm and AgNPs 1000 ppm showed a reduction in biofilm adherence of approximately 1.5-1.8 logarithmic units (Figura 34).

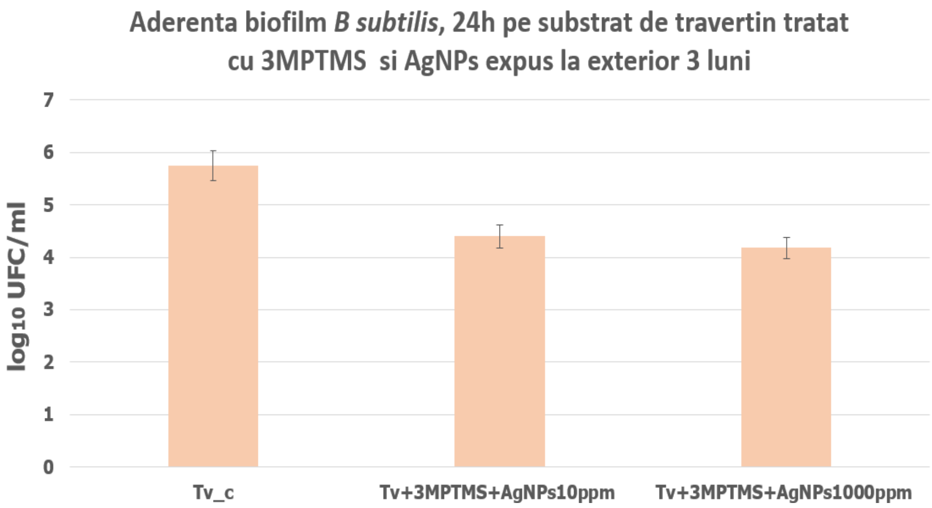


Figura 34 Graph illustrating the adhesion and biofilm formation of *B subtilis* on travertine substrate treated with 3MPTMS and outdoor exposed for 3 months

The treatment of natural stone substrates with the coupling agent Coat O Sil and exposure to outdoor conditions resulted in comparative results (Figura 35) regarding biofilm adherence regardless of AgNPs concentration (Figura 35) and the treated substrate. It is possible that the coupling agent in both cases adhered to the treated surface and allowed AgNPs to be properly fixed, regardless of AgNPs concentration and even substrate type. Therefore, the studies conducted suggest that the coatings applied to the tested natural stone samples are stable for at least 3 months in terms of antimicrobial and antibiofilm efficacy.

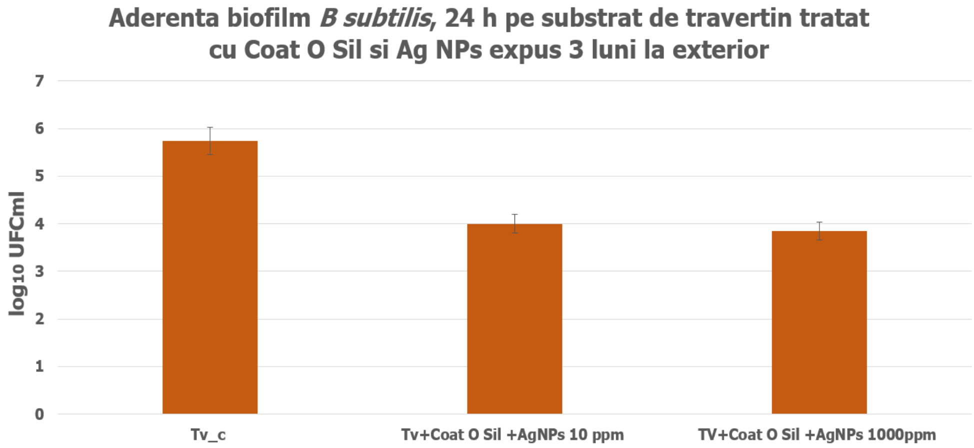


Figura 35 Graph illustrates the adhesion and biofilm formation of *B subtilis* on travertine substrate treated with Coat O Sil and exposed to outdoor for 3 months

V. GENERAL CONCLUSIONS

Chemical syntheses applied for obtaining AgNPs have led to nanoparticles with different specifications and morphology.

Chemical synthesis by room temperature reduction of AgNPs, achieved by varying the reducing agent sodium borohydride, has established an optimal ratio between the two reducing agents, resulting in NPs with sizes of up to 10 nm and stability over time.

Chemical synthesis at room temperature, although easy to apply and less costly in terms of time and energy, leads to anisotropy in the obtained AgNPs, whereas synthesis by the solvothermal method results in the formation of AgNPs with spherical shapes.

Solvothermal synthesis of AgNPs has confirmed that maintaining the temperature within the range of 200°C-220°C for 1-2 hours can lead to the formation of NPs with spherical shapes and sizes not exceeding 10 nm.

The study of antimicrobial activity for all synthesis variants has resulted in the final outcome that AgNPs obtained by the solvothermal method exhibit better activity against all tested organisms compared to suspensions of AgNPs obtained at room temperature, which show antimicrobial activity against gram-positive organisms like *S aureus*.

Tests conducted on natural stone substrates, aiming at protection against biofilm formation over time, have been performed using three types of coupling agents for surface functionalization and AgNPs fixation.

The coupling agent 3APTES forms a film on the treated substrate regardless of its porosity, while the other coupling agents are partially absorbed on the surface, maintaining the appearance of the substrate compared to the control sample. Coupling agents 3MPTMS and Coat O Sil T-cure can be recommended for treating heritage surfaces as they do not alter the appearance of the substrate.

The coupling agent 3MPTMS, with strongly reactive thiol groups towards AgNPs, has shown to better functionalize limestone or marble surfaces with medium or low porosity.

The coupling agents Coat O Sil T-cure can better functionalize highly porous structures (travertine), facilitating easier AgNPs fixation. Antimicrobial results obtained from treatments with 3MPTMS and Coat O Sil T-cure lead to relatively better values, especially for the Coat O sil agent and particularly on the porous structure of substrates.

Analyzing the Coat O Sil functionalizing agent with concentrations of 100% and 50% in a mixture with isopropyl alcohol applied on travertine and limestone substrates leads to the conclusion that a product with a polymer structure and high viscosity, such as Coat O Sil, whether used with 100% active substance or diluted, helps to fix large-sized AgNPs. This could lead to the idea that certain large-sized nanoparticles with excellent antibacterial specifications can be fixed, thus achieving satisfactory antimicrobial results.

The antimicrobial activity of AgNPs with a concentration of 10 ppm fixed on all types of natural stone is similar to the antimicrobial activity of samples obtained by the solvothermal method. Therefore, economically, AgNPs with a concentration of 10 ppm can be chosen.

Comparative analyses between different coupling agents were performed to establish the optimum type of coupling agent suitable for a particular type of natural stone substrate.

VI. PERSPECTIVE

Research will continue on selecting the appropriate coupling agent and mixing it with silver nanoparticles or nanoparticle blends to achieve a synergistic effect on treated surfaces.

The research activity will continue with the analysis of biofilm substrates, especially those originating from historical monuments and buildings requiring cleaning, biofilm removal, and protection against biofilm formation.

Research will continue with the contamination of samples with various strains or biofilms collected from different outdoor-exposed substrates, followed by treatment with AgNPs to observe the effectiveness of the studied nanoparticles.

Comparative research will continue using bi-modal or even multi-modal coupling agents to identify which coupling agents respond to the characteristics of the treated substrates, thus ensuring maximum efficacy in the coupling agent-nanoparticle system.

Research will continue by combining various types of nanoparticles to ensure both antimicrobial activity and self-cleaning properties on treated surfaces.

Research will continue by comparatively analyzing the AgNPs obtained through the syntheses mentioned in this work and AgNPs obtained on an industrial scale.

VII. LIST OF PUBLICATIONS

VII.1 Articole ISI

- ✓ **Liliana Marinescu**, Denisa Ficai, Ovidiu Oprea, Alexandru Marin, Anton Ficai, Ecaterina Andronescu, Alina-Maria Holban, *Optimized Synthesis Approaches of Metal Nanoparticles with Antimicrobial Applications*, Journal of Nanomaterials, vol. 2020, Article ID 6651207, 14 pages, 2020. (**FI 2,986**)
- ✓ **Liliana Marinescu** , Denisa Ficai , Anton Ficai , Ovidiu Oprea , Adrian Ionut Nicoara , Bogdan Stefan Vasile , Laura Boanta , Alexandru Marin , Ecaterina Andronescu , Alina-Maria Holban *Comparative Antimicrobial Activity of Silver Nanoparticles Obtained by Wet Chemical Reduction and Solvothermal Methods* Int J Mol Sci2022 May 26;23(11):5982.doi: 10.3390/ijms23115982. (**FI 5,60**)
- ✓ **Liliana Marinescu** , Ludmila Motelica, Denisa Ficai , Anton Ficai , Ovidiu Cristian Oprea , Ecaterina Andronescu , Alina-Maria Holban *A Two-Step Surface Modification Methodology for the Advanced Protection of a Stone Surface,* Nanomaterials, DOI: 10.3390/nano14010068,2023 (**FI 5,3**)
- ✓ Irina Gheorghe-Barbu, VioricaMaria Corbu, Corneliu Ovidiu Vrancianu, Ioana Cristina Marinas, Marcela Popa, Andreea Stefania Dumbrava,Mihai Nita-Lazar, Ionut Pecete, Andrei Alexandru Muntean, Mircea Ioan Popa, **Liliana Marinescu**, Denisa Ficai, Anton Ficai, Ilda Czobor Barbu *Phenotypic and Genotypic Characterization of Recently Isolated Multidrug-Resistant Acinetobacter baumannii Clinical and Aquatic Strains and Demonstration of Silver Nanoparticle Potency*. Microorganisms **2023**, vol(11), 2439 https://doi.org/10.3390/microorganisms11102439 (**FI 4,5**)
- ✓ Cornelia-Ioana Ilie, Angela Spoiala, Ludmila Motelica, **Liliana Marinescu**, Georgiana Dolete, Doina-Roxana Trusca, Ovidiu Cristian Oprea, Denisa Ficai, Anton Ficai *Decoration of a Glass Surface with Ag NPs Using Thio- Derivates for Environmental Applications.* Coatings 2024, pg 14/issue1//10.3390/coatings14010096 (**FI 3,4**)
- ✓ **Liliana Marinescu**, Cornelia Ioana llie, Denisa Ficai, Anton Ficai, Roxana Trusca, Ecaterina Andronescu, Alina-Maria Holban *Influence of nanotechnology for the treatment and protection of natural stone against biodeterioration,* Buletin Stiinfic UPB (BUL Sci) (in curs de acceptare).

Total IF 21,786 IF 13,886 articles with first author

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