POLYMERIC FILMS AND COATINGS FOR ANTIMICROBIAL APPLICATIONS

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In this paper we present the antimicrobial performances of some coatings based on acrylic polymers*. The obtained coating materials have antimicrobial properties due to the presence of silver nanoparticles in their composition. The structure and morphology of coating materials were determined by infrared spectroscopy (FT-IR) and* SEM-EDX*. The ability of silver nanoparticles to release Ag ions in an aqueous solution has been demonstrated by measuring the electrical conductivity. Film-forming characteristics and biological properties are similar, indicating that the method of obtaining the formulations is reproducible.* The introduction of 0.7% silver nanoparticles in polymeric coatings *produces an optimum improvement of the antimicrobial performances.*

Keywords: antimicrobial coatings, formulation, nanoparticles

1. Introduction

The increasing of bacterial infections is mainly due to the resistance of bacteria to various drugs. For certain species of bacteria, treatment options have become so rare that an increasing number of infections start becoming very dangerous for the patients [1, 2]. The new antibiotics are limited and preventive innovations are required to minimize the microbial pressure in infectious risk areas such as hospitals, nursing homes, etc. For example, bacteria can persist for several months on various surfaces (metal, concrete, plastic, wood), which mean a real source of contamination of the population. [3, 4, 5-7]. An innovative solution for the control of pathogenic bacteria is the creation of self-healing surface by the application of anti-biofouling coatings and / or with bactericidal properties [8]. Antibacterial coatings are interesting in health care, due to their ability of these coatings to kill pathogens. Antimicrobial coatings can contain active agents

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elution (e.g., ions or nanoparticles of silver, copper or zinc), or light-activated molecules (e.g. TiO_2).

The emergence of bacterial strains resistant to multiple antibiotics prompted the scientists and medical experts to look for alternative antimicrobial [9]. A promising alternative is the use of silver nanoparticles as a constituent of coatings for medical devices, implants and other. Researchers have shown that silver nanoparticles can be used as antimicrobial agents, but maintaining of this effect for longer periods depends on how the silver particles are fixed to the surface layer.

Because silver nanoparticles possess a large total surface area available for bacterial interaction they exhibit a stronger antibacterial effect than regular metallic silver [10]. As a consequence the incorporation of silver nanoparticles in other materials, for increasing the antibacterial capability is currently the subject of intense study. Several methods have been developed such as: silver-doped hydroxyapatite [11], polymer-silver nanoparticles [12] and silver nanoparticles on TiO_2 [13].

Almost all techniques incorporating metals in a special polymer matrix involve chemical reactions as reducing, mixing nanoparticles with polymers or more complex physical processes such as: layer by layer deposition [14], spray [sputtering] [15] or plasma deposition [16, 17, 18]. All these techniques are rather expensive and time consuming multistep syntheses that determine the complexity of the manufacturing process of materials with embedded nanoparticles.

Using the AgNPs in various medical and biotechnological applications represent one of the most intensely investigated areas. These advanced applications require chemical functionalization of the nanoparticles with organic molecules, or their incorporation into polymer matrices suitable [19]. For example the clusters of poly(acrylates) - silver nanoparticles are used for functionalization of the fibers (cotton, wool, polyester) in order to obtain antibacterial textiles [20], while the Ag / poly (EGDMA-co-AN) composites microspheres have applications as preservatives [21]. Deposition of the thin films of silver is another method of obtaining antibacterial composites. Many of the techniques presented are quite expensive and laborious.

The main objective of our research was to obtain antimicrobial coatings based on AgNPs with high toxicity both on bacteria and fungi. By covering the various substrates (concrete, wood and masonry) with this type of organic antimicrobial coatings (water-based), a dry film which effectively prevents microbial colonization through the killing of the microorganisms is created. In addition to this property, the film possesses both aesthetics and protective functions (water and disinfectants resistance - properties conferred particularly by the acrylic polymer). Because these coatings have in composition a large amount of fillers and pigments, it is difficult to introduce AgNPs uniformly dispersed on

the surface of the dried film. An inefficient dispersion of NPs in the polymeric film coatings leads to bio-degradation of the coatings. Incorporated in excess, the nanoparticles lead to an increase of the coverage density and reduces the protection performance. Excess of the nanoparticles causes insufficient wetting of NPs with polymer, resulting in the formation of a film with discontinuities and the appearance of defects in the coating system. On the other hand, a smaller amount of nanoparticles decreases the biological performance of the coatings.

Incorporating of AgNPs in coatings by using ultrasound [22] is a more expensive method and presents certain risks such as, for example, polymeric matrix degradation.

The novelty of this paper consists in approaching an efficient method for incorporating nanoparticles in coatings. Incorporating the AgNPs was achieved by a simple method, namely, grinding of the NPs with other components of the coating using pearls. This was possible by modifying the surface of the NPs with polyacrylic acid. Polyacrylic acid molecules are placed between NPs, thus preventing their agglomeration. Also, the presence of the carboxyl groups intensifies the mobility of silver ions.

To intensify the process of grinding with pearls, a spiked stirrer, was introduced inside of the mill. Using this spiked stirrer, high abrasive environments with very high viscosity can be dispersed and the possible agglomerates or clusters of AgNPs can be destroyed.

The efficient incorporation of the NPs contributes at increased antimicrobial effect even at low concentrations of the active substance (0.7wt% AgNPs).

Using this method we achieved sterile antimicrobial coatings (there is no colony of bacteria on the surface of the material - for sample 3). In our previous research [22], bacterial reduction was between 87 and 99%.

In this study, we synthesized three antimicrobial coatings based on silver nanoparticles of different sizes 15, 20 and 40 nm. Incorporating the silver nanoparticles in the polymeric material was achieved through wet grinding by using a pearl mill and an agitator (Turbo mill) in the presence of surface modifying agents. Film-forming materials were analyzed morphologically, structurally and biologically.

2. Experimental part

2.1. Materials

The acrylic polymer aqueous dispersion (41% in water), Setaqua 6756, was purchased from Nuplex. Fillers were purchased from Kronos and Titanium

Dioxide was purchased from Evonik. Polyacrylic acid was acquired from Acros Organics BVBA.

2.2 Equipments

After synthesis, the morphology and composition of antimicrobial coatings were characterized by scanning electron microscopy (SEM), energy dispersive X-ray analysis (EDX), and biological tests. EDX was performed with an accuracy of 0.5 %, with a SiLi detector from EDAX Inc. inside a scanning electron microscope model Inspect S from FEI.

Vibrational spectra were recorded using a Shimadzu 8400 spectrometer in the wave numbers range of 400-4000cm^{-1}. The ability of silver nanoparticles to release Ag ions in an aqueous solution has been demonstrated by measuring the electrical conductivity, using the Cond 330i apparatus in accordance with ISO 7888/1993 standard.

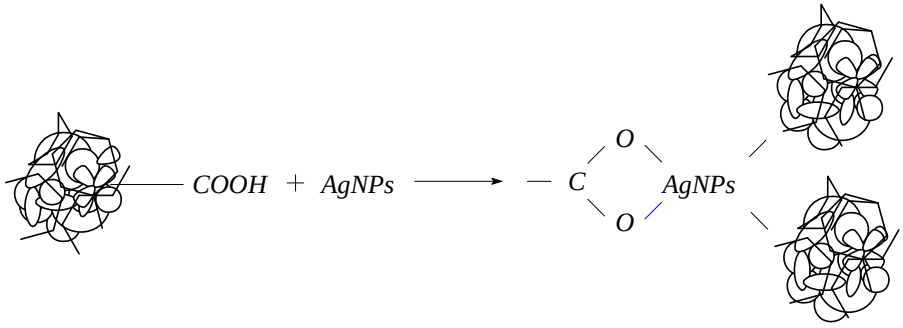
Antimicrobial Testing was done according to STAS 12719 /1989 [23] regarding "determination of the resistance to infection in mass with microorganisms of the film-forming coatings". The method has as working principle the direct contact between the film-forming material and the microorganisms' suspension, followed by checking the viability of the microorganisms.

2.3. Synthesis of antimicrobial coatings

Dispersion of the nanoparticles in a polymer material is quite difficult; nanopowders often are not wetted, or are easily agglomerated in the dispersion medium. In order to breaking up the agglomerates formed by the nanoparticles so that the fluid to become homogeneous an energy input is required. This energy can be provided by: wet mechanical milling with high shear, high pressure homogenizers or ball mills that can separate agglomerates.

The adsorption of polymeric dispersant is one of the simplest surface modification techniques to improve the dispersion stability of nanoparticles in liquid media. In terms of dispersing hydrophilic nanoparticles into aqueous media, or in organic solvents with high polarities, the anionic polymer dispersants, or the cationic polymer dispersants are widely used to generate steric repulsive forces in the polymer chains and to increase the surface charge. Thus, various types of polycarboxylic acids and their salts such as polyacrylic acid (PAA), polyacrylic acid sodium salts (PAA-Na) and co-polymers of polyacrylic acid and maleic acid are used as anionic surfactants for dispersing the nanoparticles [24].

Reactive functional groups such as -COOH can stabilize the silver nanoparticles in coatings and a homogeneous dispersion thereof may be obtained. The reaction is:



(1)

Procedure: into a pearl mill there were introduced 5g of acrylic polymer, 0.7g of silver nanoparticles (powder) and 2g of polyacrylic acid. All of these ingredients were wet milled for 40 minutes. Then were added 15.3g of distilled water, 22g of titanium dioxide (rutile) and milled for 30 minutes. Finally, there were introduced 30g filler (calcium carbonate and magnesium silicate), 20g of acrylic polymer and 5g of ethanol and were milled for 20 minutes.

With this procedure there were prepared three types of antimicrobial coating materials, whose formulations are shown in Table 1.

Table 1

Antimicrobial coatings formulations

Antimicrobial coatings	Polymer content [wt%]	Polyacrylic acid content [wt%]	Silver Content [wt%]	Silver Average diameter [nm]
Sample 1	25	2	0.7 (AgNPs1)	40
Sample 2	25	2	0.7 (AgNPs2)	20
Sample 3	25	2	0.7 (AgNPs3)	15

2.4. Film-forming characteristics

Antimicrobial coatings were applied onto glass plates using a puller (a rustproof blade provided with bumps 120 or 200 microns). The samples were placed in a well ventilated area to allow evaporation of the solvent (water). During evaporation of the solvent, the filler particles may lose their colloidal stability and particles can aggregate into gel. If the gelling begins in early stage, when the content of solvent (water) is very high, further drying of the gel leads to a mechanical stress and as result of its, cracks may appear on the surface of the film.

For a slower gelling step we tried to stabilize the fillers in coatings through:

- modification of the fillers surface by absorption of a polymeric dispersant (polyacrylate acid). This dispersant reduces the surface energy and the steric stability is achieved [24].

- adding solvents in order to decrease the concentration of the filler, the polarity level of the environment and to increase the evaporation rate. In this way the drying process time is shorter due to the rapid evaporation of the solvent. In this work, as co-solvent we had used ethanol that has a higher evaporation rate than that of water. After completion of the evaporation of the solvents (water and ethanol), the polymer molecules approach each other so much that arise the secondary valence forces. It is occurs an ordered arrangement between them and a gel is forming. Finally, it has obtained a dried uniform polymer film. The nanoparticles are embedded in the polymer film, which contributes to the increasing of both the network density and the antimicrobial properties.

2.5. Electrical conductivity

The ability of silver nanoparticles to release Ag ions in an aqueous solution has been demonstrated by measuring the electrical conductivity, using the Cond 330i apparatus in accordance with ISO 7888/1993 standard. Electrical conductivity depends on the concentration of ions, their nature, temperature, viscosity and the solvent used.

Measuring the electrical conductivity was carried out at a temperature of 25°C and neutral pH (pH = 7). Silver nanoparticles were dispersed in ultrapure water. Content in silver nanoparticles was 1000ppm.

2.6. Biological properties

The samples were used once as such and once diluted in water in a report of 1/10. The method consists in the infestation in mass of the coatings with microorganisms and determination of sterility or contamination grade of the product. For bacteria, the culture medium was simple agar and for fungus was used Agar Czapek-Dox as culture medium.

Procedure: in a Petri dish were weighed 100g of sample of coatings materials. In the weighed sample there were inoculated 0.1cm^3 of suspension of microorganisms. Then, the inoculated sample with the suspension of microorganisms is stirred with a glass rod and is maintained at a certain temperature, as follows:

- in the case of bacteria the holding time was 24 hours at a temperature of 30°C ± 2°C;

- in the case of fungi, the holding time was 72 hours at a temperature of 28°C ± 2°C and a relative humidity of 80%.

3. Result and discussion

3.1. Determination of the morphology of antimicrobial coatings

SEM and EDX images

In order to be analyzed by SEM, the antimicrobial coatings (Fig. 1) were applied onto polyethylene support, allowed to dry and finally, the obtained films were detached.

The use of the inorganic nanoparticles as additives to improve the performance of polymers is well known. To optimize the properties of the nanocomposites, a precise control of the morphology of nanostructure/nanocomposites is needed. With respect to the nanocomposites morphology, this may have an ordered arrangement of the polymeric chain and inorganic nanoparticles or may have a random distribution of nanoparticles in the polymer structure.

The morphology and the shape of surface of the material were determined by SEM analyses. When adding a small amount of nanoparticles into the polymer, the polymeric chains cover the nanoparticles. Therefore, in the SEM image, the nanoparticles cannot be visualized. At higher magnification, SEM images of the antimicrobial coatings reveal typical particles reinforced with polymer matrix composite materials. In all the images, regardless of magnification, it can see that the samples exhibited a homogeneous distribution of the particles and of the agglomerates. The agglomerates are composed mainly of calcite and titanium dioxide because they are the most abundant mineral phases and most likely they not affect the antimicrobial activity of the films.

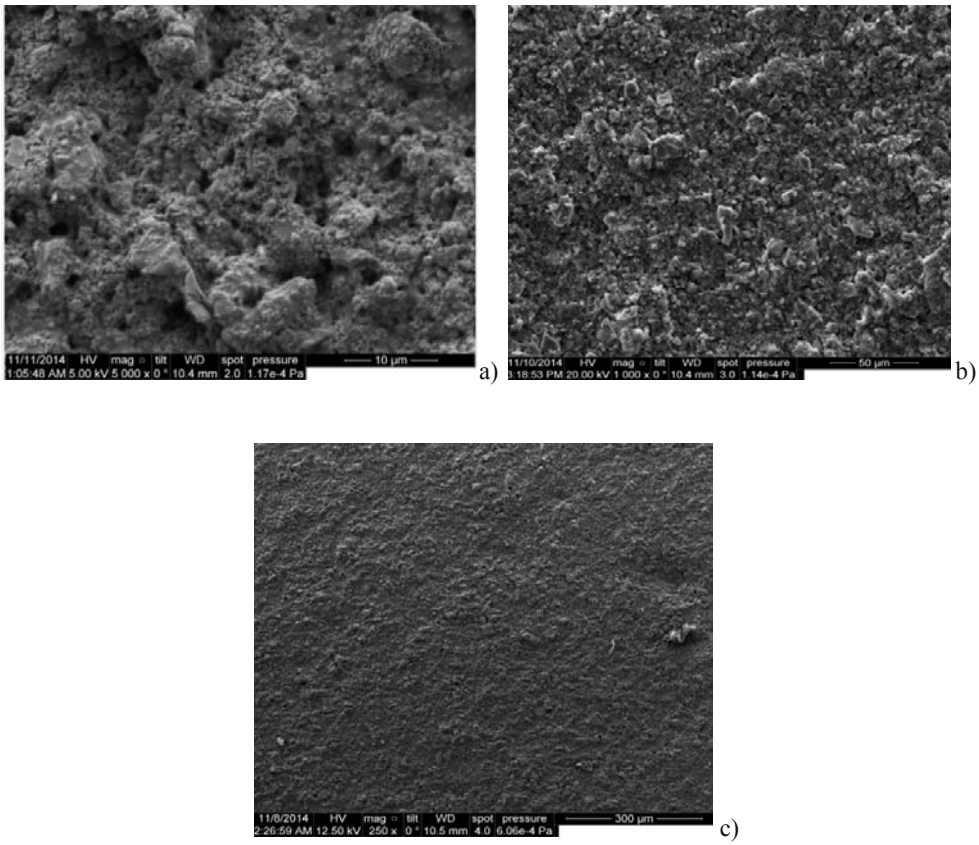


Fig.1. SEM image of antimicrobial coatings sample 3 at different magnifications: a) at 5000×: b) at 1000×; c) at 250×

Furthermore, in order to get an additional evidence of the presence of silver within the hybrid coatings an elemental analysis was obtained using the EDX technique (Fig. 2).

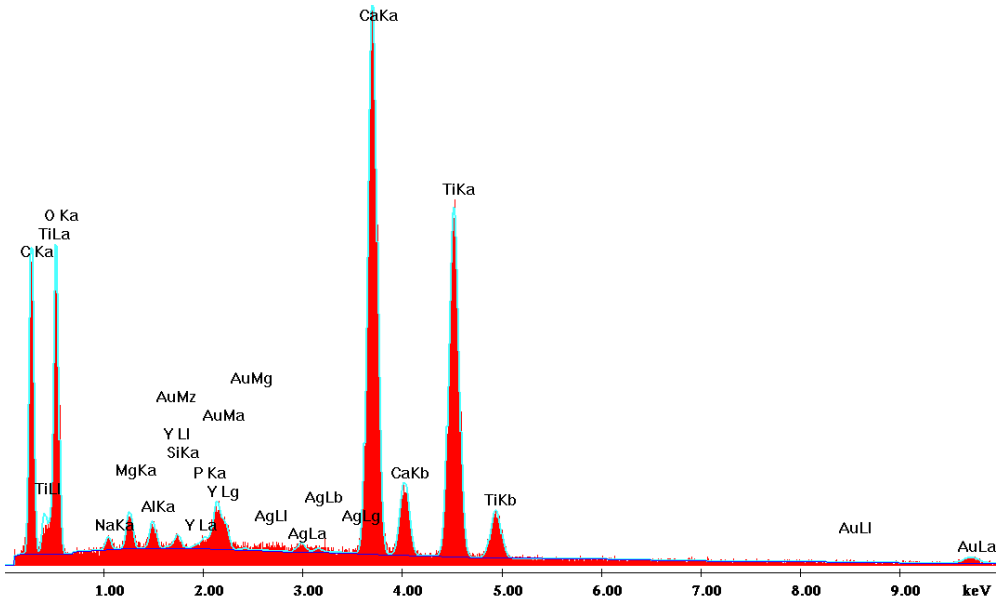


Fig. 2. EDX images of antimicrobial coatings sample 3

This EDX spectrum shows a peak at 3keV that confirms the presence of silver within the coatings. The rest of the lines of the EDX spectrum correspond to other elements present in the coatings (mainly C, O, Ca and Ti). SEM images and EDX analyses obtained from the samples confirmed the presence of silver on the surface of antimicrobial coatings. The EDX patterns indicated a small peak related to Ag, due to the low silver concentration (0.7%) (Fig. 2).

3.2. IR measurement

Vibrational spectra were recorded using a Shimadzu 8400 spectrometer in the wave numbers range of 400-4000cm^{-1}. Generally, IR spectra obtained for antimicrobial coatings synthesized (sample 1 - sample 3) are similar, indicating that the obtaining method of the formulations is reproducible. The IR spectra of the synthesized antimicrobial coatings (sample 1 - sample 3) present the characteristic peaks of the main components: titanium dioxide (rutile), $CaCO_3$ as well as the absorption bands of ethyl and methyl groups of the acrylic polymer. The spectral bands at 2924 and 2638cm^{-1} are characteristic to methyl and ethyl groups from acrylic polymer structure.

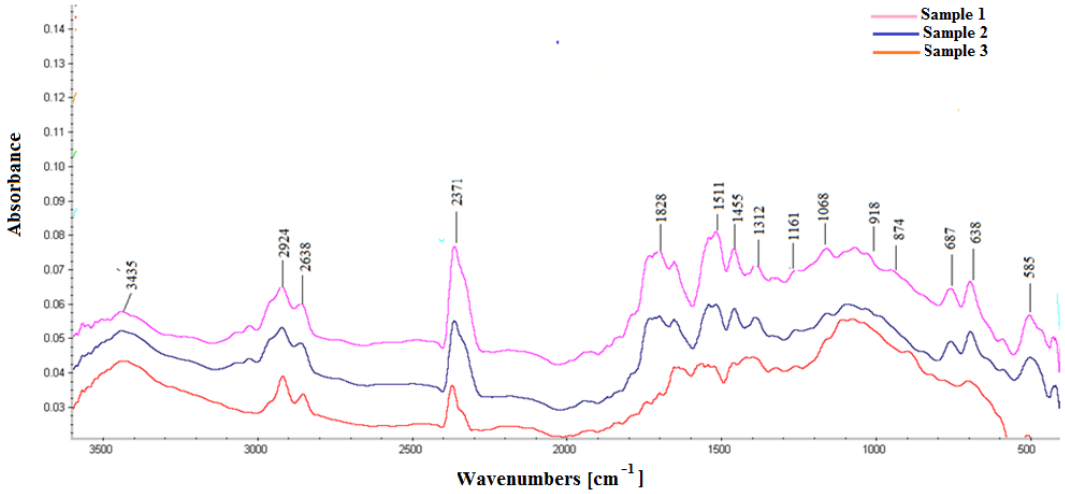


Fig. 3. IR spectra of antimicrobial coatings (sample 1- sample 3)

Spectral bands at 3435cm^{-1} are characteristic to OH groups. Therefore the spectral bands from 638 and 585cm^{-1} are assigned to the stretching and deformation vibrations, characteristic for Ti-O-Ti bond in the rutile form. Sharp spectral bands at 1828 and 874cm^{-1} are characteristic to calcium carbonate (see Fig. 3).

3.3. Film-forming characteristics

The antimicrobial coatings were characterized in agreement with the running standards and the results are presented in Table 2. It is very important that the film of antimicrobial film-forming material obtained after drying to have a good grip on the support, being well anchored by it. The formation of a continuous film is essential to ensure the adhesion, the chemical resistance and especially, the resistance to the microorganisms of the coatings.

Table 2

Film-forming characteristics of the antimicrobial coatings

Characteristics	Sample 1	Sample 2	Sample 3	Procedure
Dry film thickness 120µm [h]	4	4	4	SREN 29117:98
Appearance	continuous film	continuous film	continuous film	visual
Adhesion [N/mm^2]	0.74	0.78	0.8	SR EN ISO 4624:2003
Transmission coefficient of water vapor [g/m^{2}x h]	5.2	5.8	6	SR EN ISO 7783:2012
Transmission coefficient of water [g/m^{2}x h]	0.186	0.192	0.198	SR EN 1062-3: 2008

Determination of wet scrub resistance after 200 wash cycles. - Mass loss [g/m^2] - thickness loss [µm]	 8.2 3.15	 8.1 2.985	 8 2.725	ISO 11998:2007 Class 1
Resistance to disinfectants [24 h]	No changes on film	No changes on film	No changes on film	SREN ISO 2812-1-2007, method 3

The characteristics of the 3 antimicrobial materials are similar. Nevertheless, it can be seen a slight improvement of these characteristics which is directly proportional to the size of silver nanoparticles. As shown in Table 2, film formed after drying is uniform and has no cracks or craters.

3.4. Electrical conductivity

Metals are extremely conductive because electrons move almost with the speed of light, while in water ions move much slowly, and the conductivity is much lower. Rising of the temperature makes that the ions move faster. Conductivity is not affected by the metallic silver particles that are present in the solution, only by the silver ions. Therefore, conductivity cannot be used to determine total silver content, since the particles will not influence the reading. Pure water is an electrical insulator and the adding of the silver particles to pure water has as result that the solution still being an insulator.

Because the ions are of different sizes and carry different amounts of water when they are moving, the effect of temperature over each ion is different. It is know that the ion content in water determines its conductivity and we can use this relationship to get an indication of the concentration of silver ions that are in a colloidal solution.

The empirical relationship between conductivity and concentration of silver ions is controlled by three factors: pH, temperature and contaminating ions. The solution should have neutral pH (pH=7.0) at a temperature of 25°C and should not to be contaminated with other ions. In this case, the relationship is [25]:

$$\textit{Silver ions (ppm) = Conductivity (uS/cm) x conversion factor} \quad (2)$$

Table 3

Silver ions

Silver nanoparticles	Electrical Conductivity uS/cm	conversion factor	Silver ions [ppm]
AgNPs1 - 40nm	6.54	1.1	7.2
AgNPs2 - 20nm	6.98	1.1	7.7
AgNPs3 - 15nm	7.24	1.1	8

The conversion factor has been observed to range from 1.05 to 1.15 with an average value of 1.1.

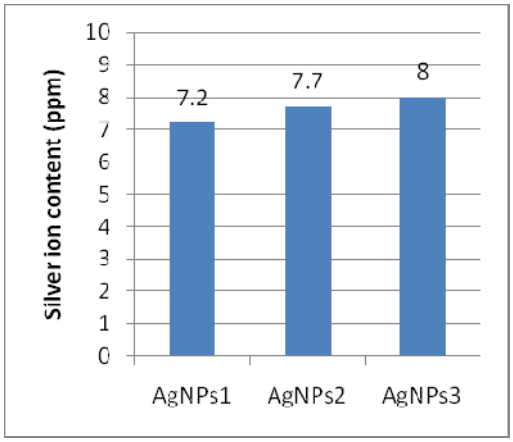


Fig. 4. Content of silver ions released from various silver nanoparticles

Silver nanoparticles (AgNPs) used in the formulation of film-forming materials releases a small amount of ionic silver (7.2ppm - 8ppm) that is beneficial for long lasting antimicrobial properties. It is observed that the ionic activity varies in a reverse versus to the nanoparticles size owing to their high specific surface.

3.5 Biological properties

Silver nanoparticles promise as antimicrobial agent, but the maintaining of this effect for longer periods depends on how the silver particles are fixed to the surface of the layer. The incorporation of silver nanoparticles in the polymer leads to a slower release of silver ions from film. In this case the polymeric material acts as a barrier against releasing of the silver ions. The efficiency and longevity of antimicrobial surface are increased because the nanoparticles are not removed immediately. It is expected that the highest concentration of silver ions to be on the surface of the coating material, respectively, the place where they are needed. It is stil unclear how this surface exerts antimicrobial activity. What it is clear is that the large surface-volume ratio is important for the antimicrobial effectiveness.

Antimicrobial activity was tested on the following strains of microorganisms:

a) Bacteria : *Pseudomonas aeruginosa* (gram negative) ATCC 9027 and *Staphylococcus epidermidis* (gram- positive) ATCC 14990.

b) Filamentous fungi: *Aspergillus niger* ATCC 16404.

Table 4

Biological properties (antifungal and antibacterial) of the antimicrobial coatings

Work strain	Sample 1	Sample 2	Sample 3	Sample 1 Dilution [1/10]	Sample 1 Dilution [1/10]	Sample 1 Dilution [1/10]
Pseudomonas aeruginosa ATCC 9027	+	+	-	+	+	-
Staphylococcus epidermidis ATCC 14990	+	+	-	+	+	-
Aspergillus niger ATCC 16404	-	-	-	-	-	-

Sterility or degree of contamination of the sample shall be determined according to the characteristics of growth and development of microorganisms and is denoted as follows: " - " no increase (sterile); '+' 1 to 10 colonies of microorganisms; '++' above 10 colonies of microorganisms; '+++' zone with confluent colonies; '++++' growing all over.

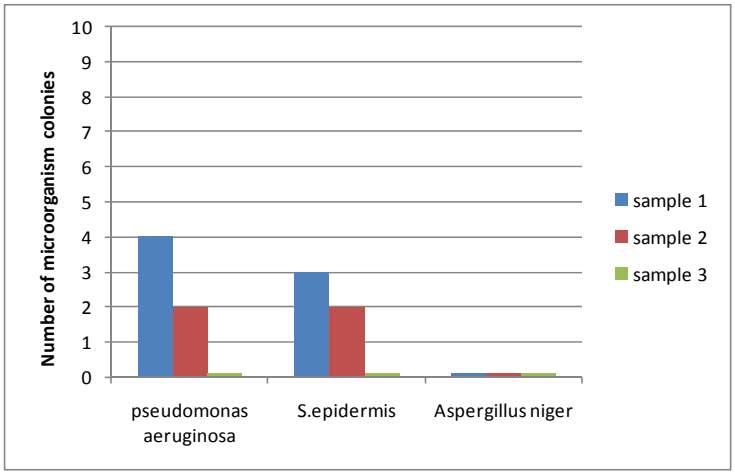


Fig.5. Antimicrobial activity of antimicrobial coatings (sample1-sample3)

Analyzing the results presented in Table 4 and Fig. 5, it is observed that the antimicrobial activity is inversely proportional to the silver content of the samples. Note that the three coating materials have antimicrobial activity even when were diluted with water. In the case of bacteria is noted the appearance of a maximum of 4 bacterial colonies on the surface of the sample. It is worth mentioning that the synthesized coatings act as an antibiotic on both on gram positive and gram negative bacteria as well as on fungi.

4. Conclusion

In this study we prepared three antimicrobial coatings based on the nano-additives (silver) by a wet grinding method in the presence of surface modifiers. SEM micrographs show a homogeneous distribution of the nanoparticles and of the agglomerates in the coating materials. The agglomerates are composed mainly of calcite and titanium dioxide because they are the most abundant mineral phases.

The presence of AgNPs and their homogenous distribution are easily pointed out by elemental EDX map recorded at 250×. Bioassays showed that the coatings have outstanding antimicrobial properties on the bacteria: *Staphylococcus epidermidis* (gram positive), *Pseudomonas aeruginosa* (gram negative) and *Aspergillus niger* (fungi). Silver nanoparticles used in the formulations of coatings release a small amount of ionic silver (7.2ppm- 8ppm) which is beneficial for long lasting antimicrobial properties. It is observed that the ionic activity is inversely proportional to the nanoparticles size owing to their high specific surface.

These data suggest that the coatings modified with silver nanoparticles is a promising material with the biological properties that may be used as an antimicrobial agent in hospitals.

Acknowledgments

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