Antimicrobial performances of some film forming materials based on silver nanoparticles

A. PICA[a*], C. GURAN, E. ANDRONESCU, O. OPREA, D. FICAI, A. FICAI
Politehnica University of Bucharest, 1-7 Polizu Str., 011061, Bucharest, Romania
[a]*Research Institute for Advanced Coating, 49 A Theodor Pallady Bd., 032258, Bucharest, Romania*

In this paper we present the biological performance of some film-forming materials with polymer matrix of acrylic-styrene type (water soluble) obtained by synthesis in solution. The obtained coating materials have antimicrobial properties due to the presence of nanosilver in their composition. The structure and morphology of coating materials were determined by Scanning Electron Microscopy (SEM), *Thermogravimetric* Analysis and Differential Scanning Calorimetry (TG-DSC). The antifungal activity of the coating materials was investigated on 12 cultures of mushrooms. Based on these results it is considered that the coating materials based on nanosilver can be used to inhibit the development of micro-organisms on concrete surfaces inside the medical units.

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Keywords: Antimicrobial, Film forming materials, Nanosilver

1. Introduction

The technological importance of nanopaticles has driven efforts to fabricate hybrid materials, which are of fundamental interest to modern science due to their vast applications in controlled drug release, drug targeting [1], inhibition of microbial biofilm growth, biosensors [2], antimicrobial therapy [3] or medical diagnostics.

In critical environments, such as hospitals and kitchens, inadvertent microbial contamination of surfaces can lead to the rapid spread of disease, initially from surface to person and than from person to person. [4]. Thus, there is a need for architectural coating compositions which dry to form solid paint films which are capable of killing or at least preventing the growth of any microbes which come into contact with the surface of the solid paint film. Developing bactericidal coatings using simple green chemical methods could be a promising route to potential environmentally friendly applications [5,6,7].

Most antimicrobial film-forming materials are based on biocides. They have a short period of antimicrobial efficacy (six months). Antimicrobial efficacy is limited to a few bacteria and proved to deteriorate over time and after repeated washing with water. It was observed that bacteria are not immediately killed once they come in contact with the surface of the coatings; their elimination takes place at 12-24 hours after infestation [8,9].

Ashavani Kumar [10] synthesize metal-nanoparticle (MNP) - embedded paint, in a single step. The naturally occurring oxidative drying process in oils, involving free-radical exchange, was used as the fundamental mechanism for reducing metal salts and dispersing MNPs in the oil media, without the use of any external reducing or stabilizing agents The surfaces coated with nanoAg based paint showed excellent antimicrobial properties against both Gram-positive human pathogens (*Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli*).

US Pat. 20100021710 describes the use of anti-microbial powders in coatings, the powder consists of metallic copper, zinc or alloys which are deposited on titanium oxide [11]. Holdar describes anti-bacterial fibers made by incorporating an anti-microbial powder, based on silver chloride deposited on titanium dioxide, in the polymer spinning solution [12].

The aim of the current paper is o proves the appropriateness of incorporating silver nanoparticles into the film forming materials.

2. Experimental part

2.1 Materials

All the chemical substances were of analytical grade. Ethyl glycol acetate provided by Poydis, dispersion agents type Pigment disperser A, S supplied by BASF, film-forming material MPAS provided by the ICAA, dioctyl sodium sulfosuccinate surfactant supplied by Sigma-Aldrich and silver nanoparticles AgNP presented in a previous paper [13].

The sample MPAS was obtained as follows: in a pearl mill water, additives and NH_3 were added and mixed for 10 min followed by the addition of the calcium carbonate and titanium dioxide and further 30 min mixing. Finally, acrylic-styrene polymer is added. The additives include surfactants, dispersants, rheological modifiers and light stabilizers.

2.2 Equipment

SEM analyses were performed on a HITACHI S2600N electron microscope operated at 25 keV, in primary electrons fascicle, on samples covered with a thin silver layer.

Differential Scanning Calorimetry (DSC) was performed with a TG-DSC STA Jupiter 449C, Netzsch at a scan rate of 10 ^{0}C/min, in argon.

2.3 Obtaining antimicrobial coating materials based on silver nanoparticles

There have been 5 antimicrobial film-forming material formulations (denoted as AM1, AM2, AM3, AM4 and AM5), using the method of synthesis in solution (Fig. 1) This method assumes the existence of a solvent (water, glycol) in which the nano silver inflates and the basis polymer of the film-forming material is dissolved. The entropy gained by removing the solvent molecules allows the polymer chains to diffuse between the particles of the nanosilver. After the solvents evaporation a dry film with antimicrobial properties is formed.

Dispersion of nanosilver particles in a polymer is quite difficult to achieve giving the phase separation or agglomeration that occurs. Studies have shown that to obtain a stable dispersion of nanoparticles in a liquid polymer, rotation radius of the linear polymer must be higher than the size of the nanoparticles. Dispersed nano particles swell the polymer chain, so the rotation radius of the polymer increases with the density fraction of the nano-particle. The initial dispersion of nanosilver particles in the film-forming material has raised some issues that were solved through the use of dispersants and effective surfactants agents.

Table 1. Antimicrobial film forming materials –AM1.... AM5

Film forming materials	Content of surfactant %	Dispersant	Content of nano Ag ppm NP Ag 1
AM 1	1	Disperser A	550
AM 2	1	Disperser S	450
AM 3	1	Disperser A	200
AM 4	1	Disperser A	300
AM 5	1	Disperser A	250

It was determined that acrylic acid polymer dispersants were excellent for stabilizing the antimicrobial in water-based formulations AM1 - AM5. Sufficient shear to disperse this material can be imparted through the use of an HSD (High Speed Disperser) such as a ULTRA TURRAX T 18 to 6000 - 30000 rpms for 1-2h. Table 1 provides the formulation of antimicrobial film-forming materials, marked with AM, based on nano Ag.

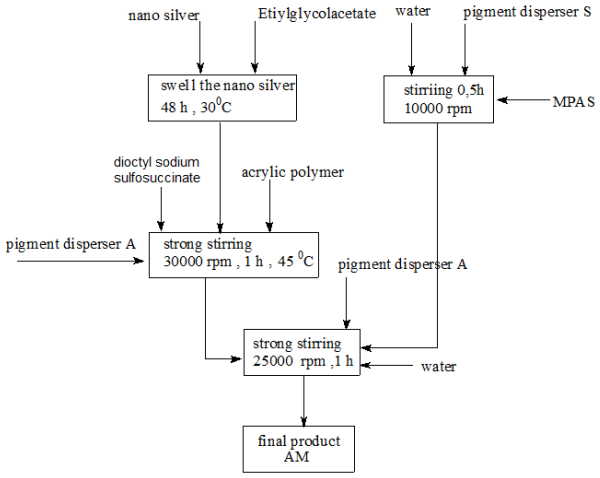
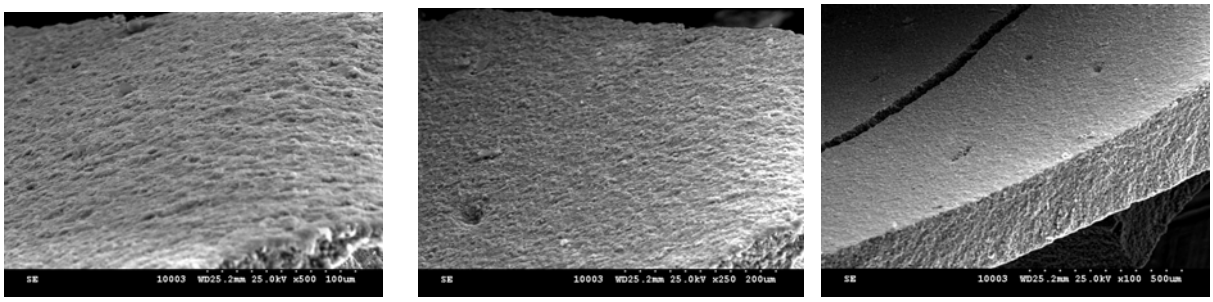


Fig. 1. Synthesis scheme for samples AM1-AM5.

3. Result and discussion

3.1 Scanning electron microscopy - SEM

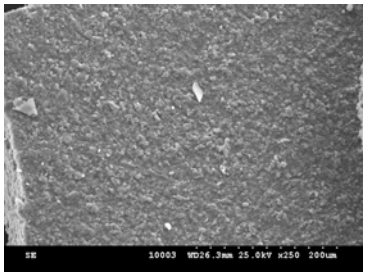
Four coating materials were selected, AM1....AM4, and were analyzed by electronic scanning microscopy. Morphologically there are important similarities between the four materials; mineral particles can be clearly identified in all cases. We can see a notable difference between the morphology surface of film-forming material and the cross section. The roughness is higher in the section than the surface. This can be easily explained taking into account the difference in density of mineral phases (TiO_2 and $CaCO_3$) from the polymer. There is a settling of these particles and an increase of the concentration of polymer on the surface. This asymmetric nature of the films is very important because the surface became smoother and consequently its penetration by different agents (water, launder, etc.) became more difficult. For sample AM1 and partially sample AM2 it is shown that the particles are visibly smaller.



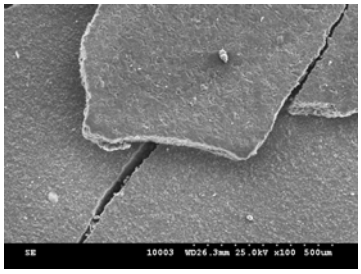
a b c

Fig. 2- SEM images of AM 1 : a-b) surface, c) in section.

a



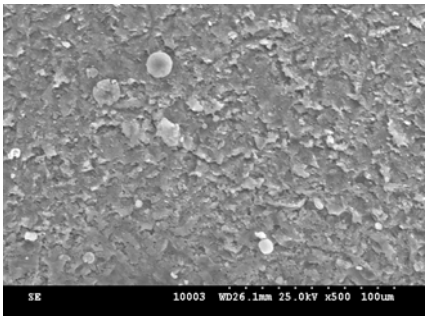
B



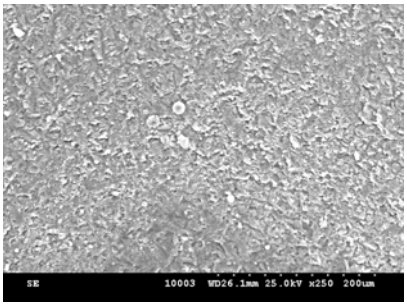
c

Fig. 3. SEM images of AM 2 : a-b) surface.

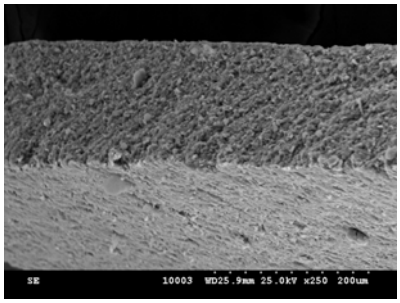
The particle size of mineral phase in the two cases is smaller than for the samples AM3 and AM4. Analyzing the material at the surface and in section we can see a better uniformity of the film-forming material in section. The explanation of the advanced homogenity of the two samples is based on the fact that sedimentation is strongly influenced by the particle size; larger particles are more likely to sedimentation.



a



b



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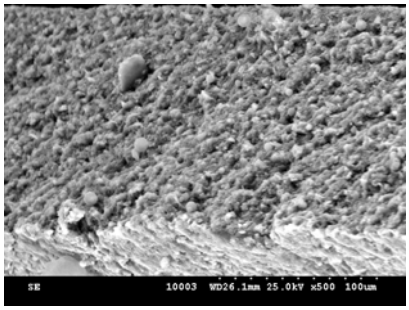


d

Fig. 4 - SEM images of AM 3 : a-b) surface, c-d) in section;

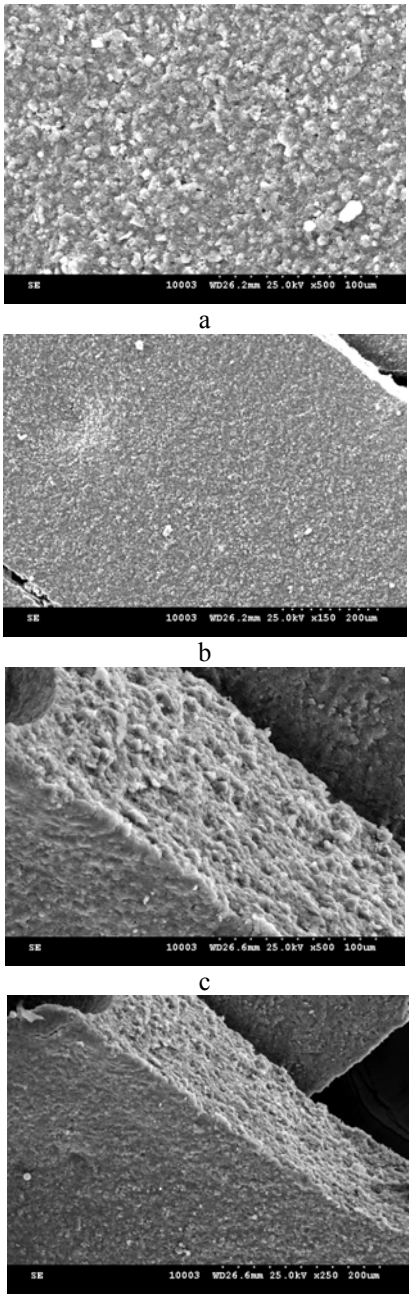


Fig. 5 - SEM images of AM4 : a-b) surface, c-d) in section

The film-forming material shows defects in the surface but these defects do not penetrate the film.

3.2 Thermogravimetric analysis: TG-DSC

The thermal analysis of sample AM1 indicates a good stability up to 250°C. The 1.1% mass loss is attributed to water and solvents molecules remaining in the structure of AM1. The mass loss is accompanied by a slight endothermic effect, -161.3 J/g. Between 250 and 495°C occurs a first degradation of sample AM1. The strong exothermic effect accompanying the process indicates an oxidative process (combustion of organic compounds in the presence of oxygen being an exothermic process while and decomposition is usually an endothermic one). This stage consists of at least two processes, partially overlapping, with 14.48% mass loss (250-385°C) and 3.51% (385-495°C), as shown in the DTG and DSC curves analysis (Fig. 6)

The last stage of decomposition, with an experimental mass loss of 21.6% was attributed to thermal decomposition of $CaCO_3$ (the remaining components of AM 1 cause the onset temperature of decomposition for $CaCO_3$ to be 700°C). The process is accompanied by a strong endothermic effect, -651.6 J/g.

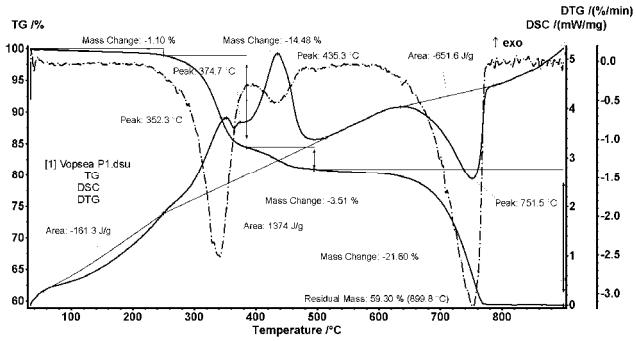


Fig. 6. DTG and DSC – sample AM 1.

For the sample AM2 the thermal analysis in terms of mass loss and thermal effects is practically identical with the thermal analysis of sample AM1 (fig.7). The values of thermal effects can vary very little between the two tests. The main difference between AM1 and AM2 samples is that AM2 has exhibit a better thermal stability, all effects being shifted with 5-10°C towards higher temperatures. Therefore, we see an improvement of the stability of film-forming material component, even the $CaCO_3$ decomposition being delayed by 10°C.

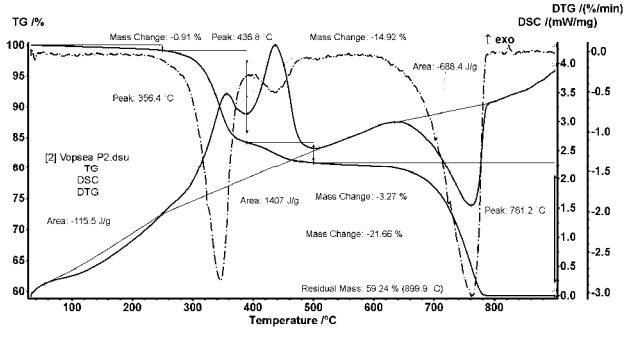
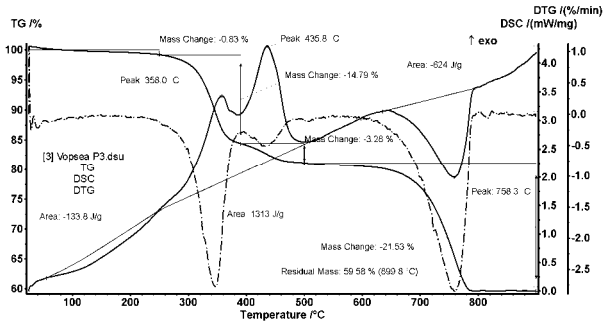
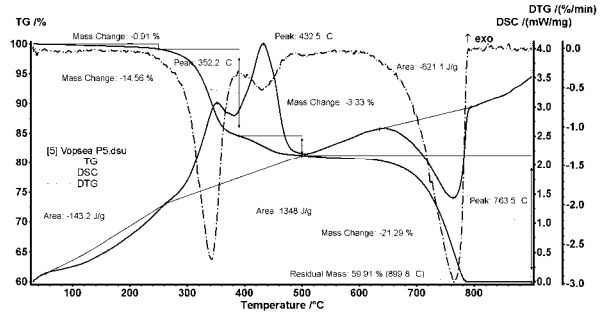


Fig. 7. DTG and DSC – sample AM 2.

For the remaining samples AM3 and AM5 – the variation of the peaks position is random probably due to the amount of nano Ag too small to be seen (fig.8).



Sample AM 3



Sample AM 5

Fig. 8. DTG and DSC – sample AM3 and AM5.

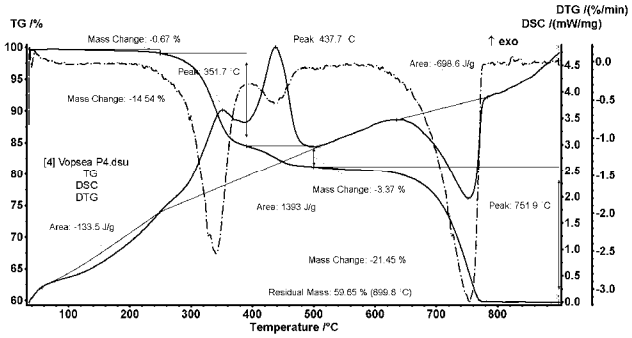


Fig. 9. DTG and DSC – sample AM 4.

For sample 6 (MPAS without nano-Ag) – it can be observed that the exothermic effect has 4 peaks, which would mean that here we can see better the separated processes of combustion (fig.10). We attributed this behaviour to the nano silver from AM1-AM5 samples, that influences the oxidation temperature of a component and practical overlaps its thermal effects.

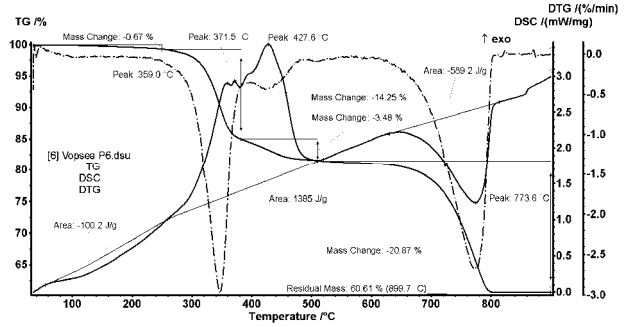


Fig. 10 . DTG and DSC – sample MPAS.

3.3 Antifugical tests

The mechanism by which the formulations AM1 - AM5 eliminate growth of the fungi begins with the controlled release of silver ions under ambient moisture. Silver ions liberated by moisture come into contact with target organisms and interact with multiple binding sites. As silver ions are transferred into the cells of target organisms, metabolic and respiratory functions of the cell cease, and, consequently, the target organism cells are destroyed.

3.3.1 Testing the AM antimicrobial coating materials on the fungal cultures

In Petri dishes, on Czapek-Dox medium supplemented with sucrose, were placed specimens of applied glass fabric with variants of AM film-forming material (2 samples in parallel) and were inoculated by spraying a mixture of spores belonging to the following fungal species: Aspergillus flavus, Aspergillus niger, Aspergillus terreus, Aspergillus fumigatus, Aspergillus versicolor, Paecilomyces varioti, Aureobasidium pullulans, Penicillium glaucum, Penicillium citrinum, Scopulariopsis brevicaulis, Stachybotrys atra, Trichoderma viride.

The antifungal testing involves three steps:

- samples were incubated in a humidifying thermostat at 30±2^{0}C and 90-100% RH of air;
- the microscopic observations were performed at 21 days after inoculation, with a stereomicroscope having a magnification of x50;
- fungitoxic effect was expressed by the size of the inhibition zone (in mm) of fungal growth around the glass fabric specimens immersed in AM.

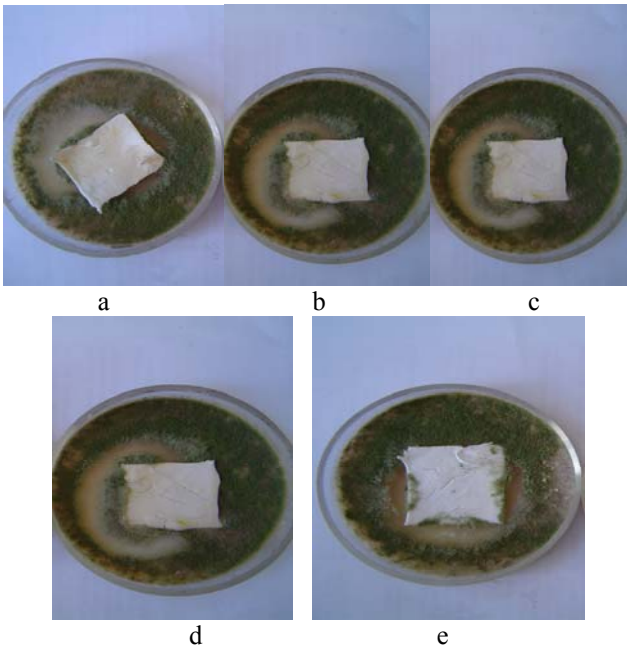


Fig. 11. a) sample AM1, b)- sample AM2, c) sample AM3, d) sample AM4, e) sample AM5 - 21 days after inoculation.

The effect of adding the anti-microbial substance to the liquid coating composition is a significant reduction in the number of fungal species surviving on the surface of the derived solid coating. This happens unexpectedly quickly, and in most cases almost all of the bacteria are killed within 24 to 48 hours. It is seen from the pictures shown in Figure 11 that all the AM1-AM5 film-forming materials have antifungal action; the antifungal effect is proportional with the nano silver content from the film-forming material. The antifungal effect holds up to 21 days.

4. Conclusions

In this paper we presented the biological performances of a film-forming material based on a polymer matrix acrylic-styrene type, water soluble and with antifungal properties due to the nano silver present in its composition.

There have been 5 antimicrobial coating material formulations AM1-AM5, based on nanosilver particles, with synthesis in solution. This method indicates the existence of a solvent (water, glycol) in which the silver nanoparticles swells and the material's polymer basis dissolves. The initial mixing of AM raised some issues that were resolved by a systematic formulation consisting in the evaluation of shear forces necessary to properly disperse the filler in the coating matrix, also the dispersant choice optimization and, ultimately, choosing the surfactants agents to ensure proper display and flow of the coating.

The structure and morphology of coating materials were determined by Scanning electron microscopy (SEM), Thermogravimetric analysis (TG-ATD) and Differential Scanning Calorimetry (DSC). It was shown that our formulations of film-forming materials with nano silver in their composition have antifungal activity in these bacteria and fungi: Aspergillus flavus, Aspergillus niger, Aspergillus terreus, Aspergillus fumigatus, Aspergillus versicolor, Paecilomyces varioti, Aureobasidium pullulans, Penicillium glaucum, Penicillium citrinum, Scopulariopsis brevicaulis, Stachybotrys atra, Trichoderma viride.

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*Corresponding author: alexandra.pica@icaaro.com