THE INFLUENCE OF SILVER NANOPARTICLES ON THE SURFACE MORPHOLOGY OF FILM-FORMING MATERIALS AND THEIR ANTIMICROBIAL EFFICIENCY

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The surface morphology was investigated, surface area, bacterial adhesion and hydrophilic/hydrophobic character of antimicrobial coating materials. The materials have a different surface morphology depending on the content of silver nanoparticles. The new antimicrobial materials show a low bacterial adherence, facts suggest that the release of Ag^+ ions by the new material could be the major cause for the low bacterial adhesion on these materials. In exchange, the surface morphology matters in the improvement of overall performance. The surface morphology was investigated by SPM and the hydrophilic nature of the material was determined by the Goniometer method. The structural parameters (surface area, pore size distribution, pore volume) were determined by BET analysis. It was demonstrated that our formulations of film-forming materials with nanosilver in their composition have antimicrobial activity at these bacteria: *Staphylococcus Aureus* and *Bacillus Cereus*.

Keywords: nanoparticles, antimicrobial, surface

INTRODUCTION

In recent years, nanomaterials have gained interest due to their unique properties (Alissawi, 2013). Composition and morphology control of NPs plays an essential part in their special applications (Liu *et al*., 2010). Nanomaterials that can be used in antimicrobial systems must be biocompatible (Podsiadlo *et al.,* 2005). The term "nanocoating" is commonly used for filling the polymer matrix with dispersed nanoparticles, having the average size below 100nm (Liu *et al*., 2010). The mixture of polymers and NPs opened the way to obtain flexible materials that have spectacular antimicrobial properties, electrical, optical or mechanical. In addition, nanoparticles can act in the polymer matrix, and can change the orientation of the polymer and its morphology, sometimes leading to micro phase separation (Menno *et al*., 2011). Silver NPs have effective antimicrobial properties, compared with other salts or silver compounds, due to the extremely large specific surface, providing better contact with microorganisms (Nedelcu *et al*., 2014).

EXPERIMENTAL PART

Materials

All the chemical substances were of analytical grade. Ethyl glycol acetate provided by Polydis, 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 presented in a previous paper (Pica *et al*., 2012).

Equipment

The SPM Ntegra Aura NT-MDT platform offers opportunities for investigating the topography in 3D imaging and physical properties of surface materials starting at the microscopic level up to the nanometer level. The structural parameters (surface area, pore size distribution, pore volume) were computed from N_2 sorption (adsorption and desorption) isotherms, obtained using Quantachrome NovaWin 1200e automated surface area analyzer. KSV CAM 101 apparatus equipped with live digital camera and special analysis software was used for static contact angle measurements performed on metallic samples.

Preparation of Antimicrobial Film Forming Materials

There have been 3 antimicrobial film-forming material formulations (denoted as AM1, AM2, AM3), using the method of synthesis in solution. This method assumes the existence of a solvent (water, glycol) in which the AgNPs 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 nanoparticles of the nanosilver. After the solvents evaporation a dry film with antimicrobial properties is formed. The method of synthesis of film-forming materials was presented in a previous paper (Pica *et al*., 2012). For antiseptic reasons, 250 –550 ppm AgNPs with particles size of 40 nm were used. The preparation and characterization of these AgNPs was presented in a previous paper (Pica *et al*., 2012).

RESULTS AND DISCUSSION

Surface Topography of Antimicrobial Film-Forming Materials

The SPM Ntegra Aura NT-MDT platform offers opportunities for investigating the topography in 3D imaging and physical properties of surface materials starting at the microscopic level up to the nanometer level. Investigations can be carried out in ambient environment or in vacuum (up to 10^{-2} torr). Figure 1 are three -dimensional images (3D) antimicrobial film-forming materials deposited on glass plates. SPM obtained studies on thin films showed very rough surfaces, with a columnar structure and uneven shapes of “valleys and hills”.

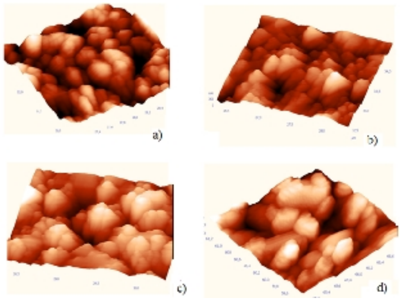

Figure 1. Surface topography of antimicrobial film-forming materials – images (3D), a) AM1; b) AM2; c) AM3; d) CM

Structures of film-forming materials are homogeneous and porous due to the tendency of nanoparticles, as development of “islands”, whose surfaces are becoming increasingly large. The images of the antimicrobial film-forming materials do not show significant differences between them, but compared with conventional film-forming material (CM), these differences are significant. Was obtained an average roughness (AR) between 306-320 nm for antimicrobial coating materials doped with AgNPs; and an average roughness of 196 for conventional coating material (CM). The results achieved confirm the studies to this point, namely that the introduction of NPs into a polymer material increases the contact surface, the surface area, pore volume and surface roughness. Rough morphology is beneficial to the film in terms of durability and antimicrobial properties, which are significantly increased.

Table 1. Average roughness (AR) of antimicrobial film-forming

Sample	AgNPs content [ppm]	Roughness [nm]
AM 1	550	320
AM 2	450	312
AM 3	400	306
CM (without silver NPs)	0	196

Surface Area and Pore Size Analysis of AgNPs - Doped Antimicrobial Film-Forming Samples

The structural parameters (surface area, pore size distribution, pore volume) were computed from N_2 sorption (adsorption and desorption) isotherms, obtained using Quantachrome NovaWin 1200e automated surface area analyzer. The samples were first outgassed at 60°C for 180 min under vacuum to 0.3 Pa final pressure, and the isotherm were measured over the P/P0 (relative pressure range) from 0.05 to 0.995 (absorption) and 0.995 to 0.05 (desorption).

Table 2. Physisorption data for AgNPs and samples AM1 -AM 3

Sample	Surface area (S_{BET}), [m^2g^{-1}]	Pore volume, [cm^3g^{-1}]	C value from BET model
AM1	13.94	0.014	3.701
AM2	11.43	0.015	4.133
AM3	11.05	0.016	4.215
AgNPs	18.33	1.91 (monolayer)	0.456

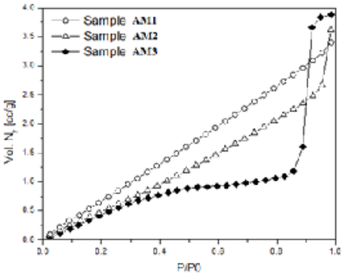

Figure 2. Nitrogen adsorption isotherm for antimicrobial film forming materials

The BET specific surface are (SBET) and the monolayer volume coverage were determined employing the Brunauer-Emmett-Teller (BET) equation.

The lack of a sharp “knee” point for the AgNPs-containing polymer samples indicates that the attractive adsorbate-adsorbent interactions are much weaker for N_2 molecules interacting with the polymer chains within the film-forming material sample, than with the Ag metal surface. The values of the C parameter of the BET equation are also reported in table 2. The C parameter is a measure of the strength of the interaction of the adsorbate (N_2) with the surface and the higher the C parameter, the sharper the ‘knee point’ in the early part of the isotherm at low P/Po.The value of C for nitrogen adsorption at 77 K on porous inorganic materials such as alumina or silica is typically in the range of 80–150 (Gregg and Sing, 1982). The C values shown in table 2 for the AgNPs samples are similar.

Contact Angle Measurements – Static Sessile Drop Method

Goniometer Method

The simplest way of measuring the contact angle is with a goniometer, which allows the user to measure the contact angle visually. The measurement of each contact angle was made for 60 seconds at 2 seconds frame interval. The contact angles reported were the mean of 2 determinations. Smaller contact angles correspond to increased wettability (hydrophilic surfaces, <90°), whereas higher values correspond to hydrophobic surfaces (>90°).

Table 3. Mean values for contact angles for the analyzed specimens

Sample	Contact angle (mean value), [°]	Comments
CM (without silver nanoparticles)	70.5	<90°, low hydrophilic
AM1	85.4	<90°, low hydrophilic
AM2	85.1	<90°, low hydrophilic
AM3	84.8	<90°, low hydrophilic

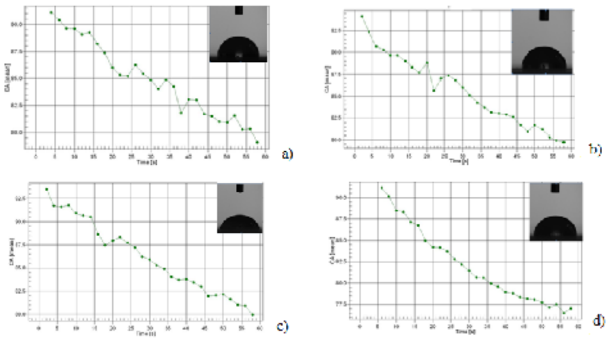

Figure 3. Variation of the contact angle in time for the: A – AM1 sample; B – AM2 sample; C – AM3 sample; D – CM sample

The samples were homogeneous in composition along the surface. Figure 3 shows the variation of the contact angle in time for the CM sample and AM1-AM3 samples using as reference liquid water. The samples AM1-AM3 show no significant differences in terms of contact angle values, and all of them have a higher value for the contact angle as compared to the CM (see table 3).

Antimicrobial Tests

Surface load of bacteria varies with the species of bacteria and is influenced by the growth environment, pH and ionic strength, age of bacteria, and surface structure of the bacteria. However, the relative contribution of bacterial surface load to bacterial adhesion was not clearly understood. For this reason we preferred to get a slightly hydrophilic antimicrobial material which retains a small amount of water. The bacteria are carried on the coating material by moisture (water). In return, moisture activates the silver nanoparticles which release silver ions. The resistance of the antimicrobial coatings to mass infection with the micro organisms was determined according to STAS 12719. This method is basically the direct contact between the AM antimicrobial coatings and the suspension of microorganisms. The AM1-AM3 film forming materials are applied on filter paper rings, dried and inserted into the culture dishes with micro organisms.

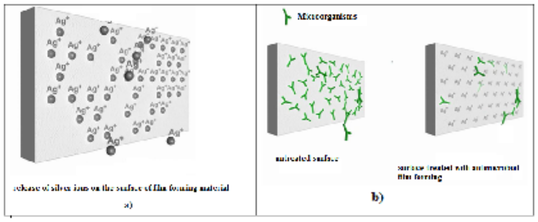

Figure 4. The mechanism by which a film-forming material exhibit antimicrobial effect: a) release of silver ions on the surface of film-forming material, b) the untreated surface is full of microbes and the surface treated with antimicrobial film-forming material destroys the microbes

Figure 4 shows schematically a surface treated with film-forming material that releases silver ions on the surface compared with untreated surface that is contaminated with germs. As you can see the surfaces treated with antimicrobial film-forming material, suppress microorganisms and won't let them grow. An active layer of film-forming material may be effective only if the active substance is released. Antimicrobial activity of a coating containing silver depends on the concentration of Ag^+ released. As shown in Figure 5, all the coating formulations (denoted as AM1- AM3), have antimicrobial activity, with a inhibition capacity between 87-99% (*Staphylococcus Aureus*) and 85- 98% (*Bacillus Cereus*) and the blank sample marked with CM (without silver nanoparticles) does not have antimicrobial activity. The nanosilver content positively influences the antimicrobial activity; the best results were obtained by the sample AM1, in which the AgNPs content is 550 ppm.

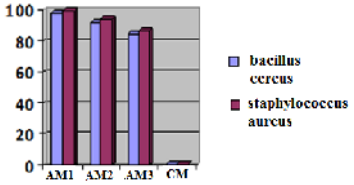

Figure 5. Growth inhibition of AM1- AM3 antimicrobial film-forming materials against *Staphylocococcous Aureus* and *Bacillus Cereus*

CONCLUSIONS

The results achieved confirm the studies to this point, namely that the introduction of nanoparticles into a polymer material increases the contact surface, the surface area and pore volume and also the surface roughness and its hydrophilic character. The roughness morphology is beneficial for the particle in terms of sustainability and antimicrobials properties, which are significantly increased. It was shown that our formulations of film-forming materials with nanosilver in their composition have antimicrobial activity at these bacteria: Staphylococcus aureus, and Bacillus cereus.

Acknowledgments

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