# Studies on morphological and structural stability of some antimicrobial film-forming materials, in time

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Film-forming materials described in this study may be used for long term antimicrobial protection of wood, concrete, gypsum boards and other building materials. The materials have antibacterial properties due to the presence of AgNPs (silver nanoparticles) in their composition. The material has the ability to form a nanomodified polymer matrix which generates in the styrene acrylic binder, nanostructured configuration. This gives the composite material properties, feature and superior performance impossible to achieve at a micro scale. AFFM (antimicrobial film-forming materials) were analyzed of structure by FT-IR (Fourier transform infrared spectroscopy) and in morphologically by electron microscopy (SEM/EDX). The introduction of (0.1-0.2)wt%. AgNPs in AFFM produces an optimum improvement in the antibacterial resistance. It has been shown that the new material (AFFM) does not deteriorate even if subjected to an accelerated aging process.. Materials were applied inside a medical unit and found that after 6 months after the application, morphology, structure and biological properties haven't undergone significant changes. Energy dispersive X-ray analysis (EDX) confirmed that AgNPs are well anchored in film-forming material, even after 6 months.

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## 1. Introduction

The World Health Organization, in a study in 2012, predicted, at world level, that over 30% of the complaints were related to indoor air quality of new and refurbished buildings [1]. For these reasons, there is currently a high demand for antimicrobial coatings to control the growth and spread of bacteria which can be the cause of the poor indoor/outdoor air quality. Covering surfaces with microorganisms can cause bacterial infection of the people who come in contact with them. The emergence of colonies of microorganisms on the surface of the organic coatings leads to degradation of them. Today, the focus is on the development of antimicrobial nano coatings efficient to a large number of microorganisms, giving the surface a long-lasting antimicrobial protection. Nanomaterials are part of the nano-coatings; the organic substances (binder) are doped with metallic nanoparticles. They are the current trend to develop new nanostructured materials. The antimicrobial coatings based on silver nanoparticles show great interest because of the antibacterial efficiency of the silver over the 650 pathogens and the maintaining for long time of the antimicrobial activity. Reduced silver extremely small (nano) leads to a number of important benefits that are not found in silver micro. The first relates to antimicrobial performance. The key to optimizing the use of silver nanoparticles as antimicrobial agent is to maximize the release of silver ions targeting the elimination of microbes [2]. Use of other solutions based on silver (various salts or compounds) involves the use of larger amounts of silver to be effective [3]. The emergence and development of silver nanoparticles of various sizes and shapes have led to increasing the use of silver as a biocide [4]. Researches to date have demonstrated that nanoscale silver shows good antimicrobial activity but is not fully understood how it works. It is very important that these nanoparticles be used correctly so that infections caused by various bacteria or fungi can be eliminated [5,6,7.8]. The silver worked best when moisture was present [9]. Colloidal stability is determined by Van der Waals type interactions, electrostatic and steric [10]. To avoid agglomeration of nanoparticles are generally used two methods: electrostatic and steric stabilization. The electrostatic stabilization ensure the existence of the electrostatic repulsion forces through the use of compounds that can have an electrically charged surface when brought into contact with a polar medium [11]. Steric stabilization implies the existence of steric forces. The surfactants can be adsorbed on the surface of the nanoparticles so that the lipophilic chains extend into the solvent, thus interacting each other [12]. Studies by Peleg and Rosenthal shows that intra-hospital infections are one of the major causes of death in Europe and America [13,14]. These nosocomial infections are caused by these bacteria, fungi and viruses [15]. For a film-forming material to be efficient in terms of biological activity, reducing the bacterial population on the stand must be done in short time. These bioassays were conducted only in laboratory conditions immediately after the obtaining operation. What happens to these materials in time is not yet fully elucidated. There are many

unknowns in terms of amount of silver that is released from material and the duration of antimicrobial effect.

In this study we aimed to investigate these issues, given that antimicrobial film-forming material obtained by us was applied inside a hospital unit. It was aimed at reducing the amount of silver in the film-forming material composition. This was achieved by optimizing the experimental conditions. We studied the influence of the content of AgNPs on the degradation of the film-forming material through accelerated aging tests. Was investigated structure, morphology and biological properties of AFFM after 24h and 6 months after their application in a medical unit. EDX quantitative analysis confirmed that there is significant releases of silver from film-forming material after 6 months.

## 2. Experimental part

### 2.1. Materials

The raw materials used in the formulation of AMFF have been: $TiO_2$, dispersing additive and defoamer by Evonik, aqueous dispersion of a nonionic stabilized thermoplastic styrene-acrylic polymer supplied by BASF, and calcite by CHHAVI Microfine Products. As a biocide for package and for film, were used silver nanoparticles (powder) with a mean particle size of 15 nm.

### 2.2 Equipment

**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, at an acceleration voltage of about 20kV, on samples covered with a thin gold layer.

**FT-IR spectra** were recorded using a FTLA 104-2000 ABB spectrometer in the field 750 –4000 $cm^{-1}$.

**Exposure of coatings** to artificial aging was performed with ATLAS UV 2000 device.

**The antibacterial activity** of AMFF sample was assessed according to the ISO 27447 standard. Film-forming materials were applied on the polyethylene film (glued on plasterboard panel) and were left for 6 months inside a medical unit. At 24 h and 6 months after application, biological tests were carried out regarding the resistance of *Staphylococcus aureus* (ATTC 6538), Gram-positive) and *Escherichia coli* (ATTC 10536), Gram-negative) both cultured in Nutrient Broth Agar. Were cut samples with sizes 2x2cm, then were placed in Petri dishes and inoculated with microorganisms.

### 2.3 Obtaining the antimicrobial film- forming materials

The literature presents only a few structures of film-forming materials doped with nanopowders with antimicrobial properties. The polymers used are of alkyd, polyurethane, and vinyl type. The most techniques incorporating metals in special polymer matrix involves chemical reactions as reducing, mixing nanoparticles with polymers or more complex physical processes such as: layer by layer deposition [16], spray [sputtering] [17] or plasma deposition [18]. All these techniques take time, costs, multistage synthesis which determines the complexity of the manufacturing process of materials with embedded nanoparticles.

For this reason I preferred to use a simpler process for introduction of the AgNPs into the film-forming material, which to ensures efficient dispersion of the nanoparticles. Direct introduction of silver in film-forming material and its agglomeration leads to decreased resistance to microorganisms. Film-forming material may be contaminated with microorganisms both in packaging and in the dry film. The attack of microorganisms can cause odors, rust, stains and damage the film.

AgNPs were first subjected to the process of swelling in a solvent miscible with water, after which they were introduced in the synthesis process of the AFFM. The amount of AgNPs is very small and if they are not well dispersed in the film-forming material, its biological function is reduced. I preferred to reduce the amount of silver because of its high price, although without affecting the biological properties of AFFM. The manufacturing process takes place in two stages, in aqueous medium:

**Swelling AgNPs in ethylene glycol**.

In a glass vessel were placed 5mL AgNPs powder and 0.2ml sodium polyacrylate, over which were dripped over 1 hour 20 ml of ethylene glycol acetate. The mixture was kept in the dark for 5 hours under magnetic stirring. In order to accelerate the wetting of AgNPs we introduce 0.3mL siloxane EFKA 3778 (wetting additive). This additive is adsorbed on the surface of nanoparticles and maintains adequate distance between polymer and nanoparticles through steric stabilization, thereby reducing uncontrolled flocculation tendency. Finally it was obtained a brown colloidal solution, homogeneous. Silver concentration in the solution was 19.6%.

**Getting antimicrobial coatings materials**

In a vessel equipped with stirring, type Cowless, were added 100 mL of distilled water, 8 mL sodium polyacrylate and different amounts of AgNPs swollen. Mixing for 10 minutes. 400g styrene acrylic polymer, 250g calcite, 170g titanium dioxide and 180ml of distilled water is added . The stirring was continued for 2 hours.

Formulation of antimicrobial film-forming material achieved are presented in Table 1.

The film-forming materials were applied on a plasterboard or polyethylene substrate and were placed inside a hospital unit. The application was performed using an applicator (a rustproof blade provided with bumps of 120 microns).

*Table 1. Preparation of antimicrobial film-forming materials AFFM*

| Film forming materials | Dispersing additive Content [wt%] | Wetting additive Content [wt%] | Dispersion time [h] | AgNPs content [wt%] |
|---|---|---|---|---|
| AFFM 1 | 0.8 | 0.5 | 2 | 0.1 |
| AFFM 2 | 0.8 | 0.5 | 2 | 0.15 |
| AFFM 3 | 0.8 | 0.5 | 2 | 0.2 |

## 3. Result and discussion

Nano modified material generates nano-structured configurations that provide exceptional properties compared with conventional products based on micro-scale compounds. The excellent durability of this nano modified material, improved protection properties, high resistance to mechanical shock, wear, oxidation, weathering, antimicrobial, is the result of nanoparticles. Properties of nano modified film forming based on styrene acrylic polymer are possible in the conditions of a correct spacing of nanoparticles in the polymer matrix by binding them in the binder polymer.

### 3.1 Fourier transform infrared spectroscopy of antibacterial film forming materials

FT-IR spectroscopy consists in determination of the functional groups of the sample analyzed. Functional groups absorb IR radiation at characteristic frequencies. FT-IR spectroscopy is a conventional method to elucidate the structure and to identify a chemical compound. Infrared absorption spectra (IR spectra) are vibrational spectra of the molecules [19]. Interpretation of the FT-IR spectrum involves the correlation of the absorption bands of the absorption spectrum of unknown compound with the absorption bands known for each type of chemical bond.

The FTIR spectra of the synthesized AFFM1–AFFM3 and CM (without AgNPs-was conducted under the same conditions as AMFF) present the characteristic peaks of the main components: styrene acrylic polymer and $CaCO_3$. Spectral bands at 1084-797cm$^{-1}$ were assigned to the stretching vibrations v1, v2 and to the bending vibrations v3, v4, characteristic of the structure of calcium carbonate [20]. The fundamental bands of calcite can be seen at: 708cm$^{-1}$ (bending vibration), 883cm$^{-1}$ (stretching vibration) and at 1400-1500cm$^{-1}$ (bending vibration) [21]. The typical IR bands of styrene acrylic polymer appear at 2850-2970cm$^{-1}$, 2100-2150cm$^{-1}$ (aliphatic CH), 1712 -1750cm$^{-1}$ (carbonyl), 1450-1650 cm$^{-1}$ (aromatic ring breathing) [22]. These absorption bands are due to aromatic skeletal "ring breathing" vibration and are extremely helpful in detecting the presence of styrene in any polymer. OH groups appear in the range 3200-3380cm$^{-1}$ and carboxyl groups occur at 1330-1512cm$^{-1}$ (see Fig. 1).

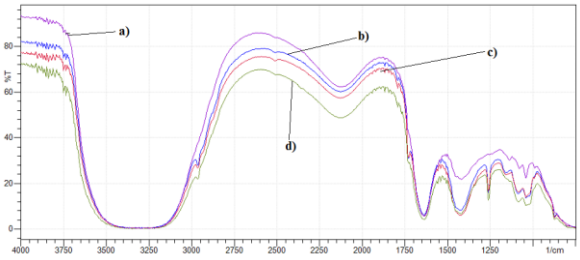


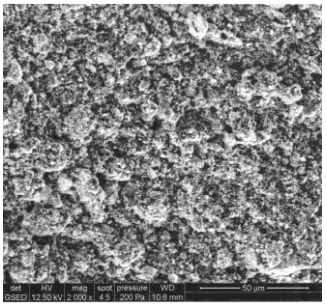

*Fig. 1. Fourier transmitter infra spectroscopic analysis of film-forming materials after 24h at application a) CM (without AgNPs); b) AFFM1; c) AFFM2; b) AFFM3*

Analyzing the FT-IR spectra of these materials, note that there are no significant changes at different concentrations of AgNPs; pick values are alike, the only difference is decreasing the area Pickle and the increasing the AgNPs content. The explanation may be that uptake of nanoparticles on the surface of the film-forming material, change spectral properties.

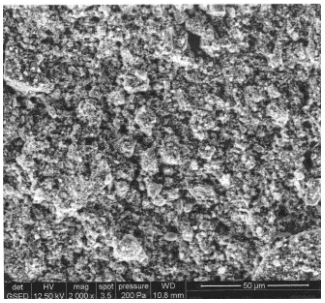
### 3.2. Morphological characterization of film forming materials SEM and EDX images

In general, researches till to date has focused on studying the morphology and structure of film-forming material immediately after synthesis in laboratory conditions. What happens with these materials being in operation in infectious environments is not fully known. It is possible that during exploitation, AgNPs to detach themselves from material and to occur defects on surface (craters, exfoliation). There is a risk that the entire amount of AgNPs to be released from the film when the film-forming material is cleaned with water or detergent, the method often used in hospitals. If AgNPs are removed in the wash water in large amounts can cause problems for the environment. For SEM and EDX analysis, the film-forming materials were applied on polyethylene film and were left for 6 months inside the medical unit.

To perform these analyzes were cut samples with sizes 2×2cm. After 6 months of the application was observed as film-forming materials do not show changes or detachment of the film. The dry film is continuous, without defects or blemishes. SEM micrographs are similar and has no changes after 6 months of application. For this reason we have presented here, only the SEM micrographs of AFFM3 at 24h (Fig. 2a) and 6 months (Fig. 2b) from application.



*2a)*



*2b)*

*Fig. 2. SEM image for AFFM3; a)- after 24 hours from application; b)-after 6 months from application*

SEM analysis was also carried out in order to evaluate the morphology of the applied film-forming materials. Fig. 2 shows that the styrene-acrylic film-forming materials presents a micrometric substructure, due to the water-based materials formulation without any surface holes or significant roughness, irregularities and cracks.

Morphologically there are important similarities between AMFF3 (after 24Hh and AMFF3 (after 6 month), mineral particles can be clearly identified in all cases. After 6 months such materials have not undergone significant morphological changes. Uniformity can be explained by dispersing efficiency of silver nanoparticles and other components (fillers, pigments) in the film-forming material.

**EDX analysis**

Highlighting quantitative components of film-forming material was performed by EDX analysis. Spectra of film-forming materials are similar and that is why we have presented here the AFFM3 sample spectrum (Fig. 3).

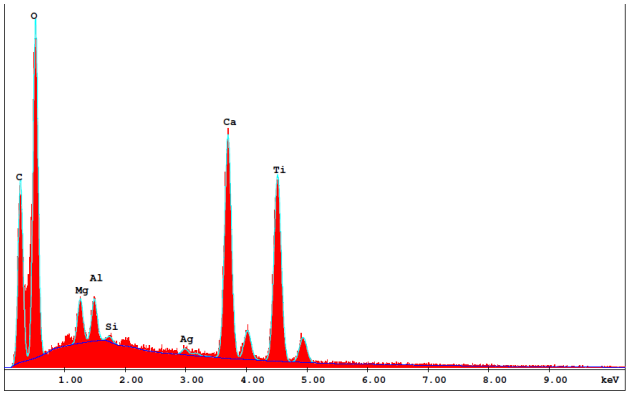



*Fig. 3. EDX spectrum of the sample AFFM3*

Table 2 shows the main elements of AFFM quantitative after 24h and 6 months from application.

According to data obtained by EDX analysis, there is a silver leaching if AFFM3 sample of 4.2% after 6 months. It is possible that this leaching to be higher or lower due to instrument error, especially as the amount of silver is very small. The instrument error is 1% for heavy elements and 2-3% for light elements (C, O).

*Table 2. The main elements of AFFM (by EDX analysis)*

| Sample | C [wt] | O [wt] | Ca [wt] | Ti [wt] | Ag [wt%] |
|---|---|---|---|---|---|
| **after 24 hours from application** | | | | | |
| AFFM1 | 26.21 | 29.4 | 26.45 | 17.55 | 0.12 |
| AFFM2 | 26.61 | 29.16 | 25.89 | 17.8 | 0.16 |
| AFFM3 | 25.81 | 29.05 | 26.76 | 17.35 | 0.22 |
| **after 6 months from application** | | | | | |
| AFFM1 | 26.14 | 29.1 | 26.25 | 17.05 | 0.12 |
| AFFM2 | 26.48 | 28.91 | 25.6 | 17.31 | 0.16 |
| AFFM3 | 25.49 | 28.95 | 26.4 | 17.01 | 0.23 |

### 3.3 Antibacterial properties

Silver ions bind with the proteins produced by bacteria (negatively charged), disabling the bacteria [23]. Between hydrophilic polar groups and water molecules, also polar, take place strong interactions which determine the hydration of these groups within the aqueous phase. Because of this the slightly hydrophilic materials have a better stability of the nanoparticles /polymer.

Film-forming materials were applied on the polyethylene film and were left for 6 months inside a medical unit. At 24h, and 6months after application, biological tests were carried out regarding the resistance of bacteria.

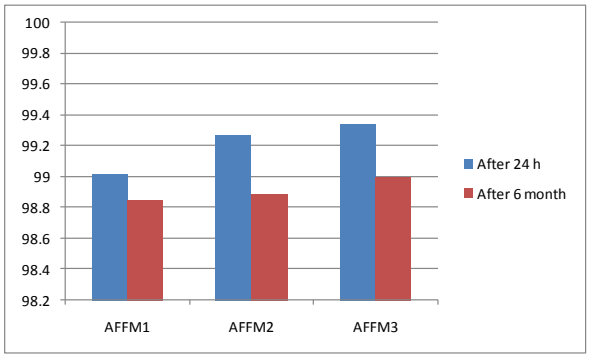
The antibacterial activity of AFFM sample was assessed according to the ISO 27447 standard, as follows:

-Petri dishes containing a moisture control paper filters, glass rods to avoid contact between test pieces and filter papers.

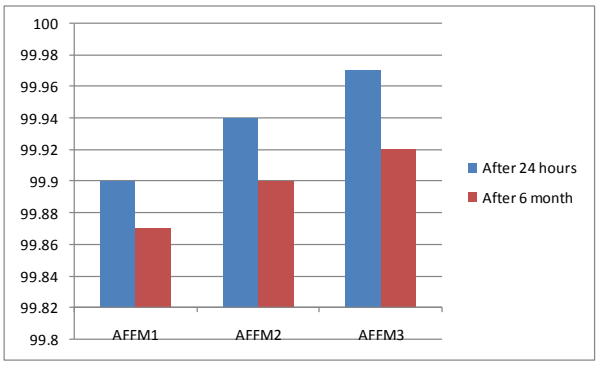
-according to the dimensions of samples, specimens were inoculated with 0.038 ml of a bacterial suspension of ca 106 CFU/ml

-clear adhesive films of 2 x 2cm were placed over the test samples.

It was determined antimicrobial resistance of AFFM at *Staphylococcus aureus* (ATTC 6538), Gram-positive) and *Escherichia coli* (ATTC 10536), Gram-negative) both cultured in Nutrient Broth Agar.




*4a)*




*4b)*

*Fig. 4. Microbial population Percent Reduction of AMFF - a) on Staphylococcus aureus (Gram-positive); b) on Escherichia coli (Gram-negative)*

Results demonstrate that there was a significant reduction of bacterial populations for 2h. The antibacterial activity was higher on *E. coli* with respect to *S. aureus*. AFFM3 samples induced the reduction (99.97%) on *E. coli* after 2h exposure (Fig. 4b).The antibacterial activity seems to correlate with content of AgNPs (samples AFFM1 and AFFM2), show lower activity than AFFM3. Note that after 6 months the antimicrobial activity remains at high levels. These results correlated with EDX results confirm that the AgNPs are well anchored in the film-forming material and the biocide activity retains at least 6 months. AFFM3 has the best antimicrobial activity, after 6 months, although according to EDX, there is a silver leaching of 4.2%. We suppose that the silver leaching is less in reality because of the error of the instrument or this loss is real and the antimicrobial properties are influenced by the content of silver (AFFM3 has greatest content in silver).

### 3.4 Stability in time

Accelerated laboratory methods, seeks to intensify the effects of environmental factors, so that damage to occur faster than occurs naturally. AFFM1-AFFM3 were subjected to artificial aging to demonstrate long-term effectiveness. Artificial aging test was performed by determining the resistance of the dry film to UV, according to EN ISO 11507. Accelerated aging test was performed with a ATLAS UV 2000 device. Photodegradation occurs within days or weeks that in the external environment may take place in years. It also has a simulation of moisture, so that materials can be tested under controlled humidity. Antimicrobial film-forming material (AFFM1-AFFM3) were applied to the wood specimens. The samples were placed in the apparatus and were subjected to 10 cycles of aging. One cycle contains 4 hours - irradiation, 4 hours - spraying and 4 hours - condensation. The results are presented in Table 3.

*Table 3. Testing antimicrobial coatings materials marked with AFFM1-AFFM3 to accelerated aging*

| Sample | Coat after 2 cycles | Coat after 4 cycles | Coat after 6 cycles | Coat after 8 cycles | Coat after 10 cycles |
|---|---|---|---|---|---|
| AFFM1 | smooth coat | smooth coat | smooth coat | smooth coat | smooth coat |
| AFFM2 | smooth coat | smooth coat | smooth coat | smooth coat | smooth coat |
| AFFM3 | smooth coat | smooth coat | smooth coat | smooth coat | smooth coat |
| CM (no AgNPs) | clear film, without changes | clear film, without changes | clear film, without changes | clear film, without changes | opalescent film slightly yellowed |

As seen from the data presented in Table 3 it seems that the introduction of Ag NPs in AMFF influences aging resistance and clarity. As for CM one can notice a slight yellowing of the film probably because of degradation of styrene polymer. Opalescence can be attributed to a slight sedimentation of the calcium carbonate. AFFM1-AFFM3 samples showed no defect on surface, the film is smooth and uniform even after 10 cycles of artificial aging.

## 4. Conclusion

Three antimicrobial materials AFFM1-AFFM3 with 0.1, 0.15 and 0.2wt% silver nanoparticles were obtained. Analysis of scanning electron microscopy (SEM-EDX) and FT-IR reveal the structure and morphology of the coating materials. Bioassays have demonstrated antibacterial efficacy of the coating materials against *Staphylococcus aureus* and *Escherichia coli.* Artificial aging tests have shown that the introduction of AgNPs in film-forming material influence aging resistance of the film and its clarity, beneficially. SEM and XRD analysis confirmed that the morphology and structure of film-forming material does not change after 6 months of application. According to data obtained by EDX analysis, there is a loss of silver in film-forming material of max 4.2% after 6 months. Biological tests have confirmed that the antimicrobial activity of film-forming materials is maintained at high levels even after 6 months.

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## References

[1] G. Carp., L. Daina, P. Armean, Analele Universitati din Oradea, **XII/B**, 57 (2013).
[2] I. A. Nedelcu, A. Ficai, M. Sonmez, D. Ficai, O. Oprea, E Andronescu E, Curr. Org. Chem. **18**(2), 173 (2014).
[3] A. Mathiazhagan, R. Joseph R, Int. J Chem. Engineering and Applications. **2**(4), 225 (2011).
[4] M. Rai, A. Yadav, A. Gade, Biotechnol. Adv. **27**, 76. (2009).
[5] T. Nakane, H. Gomyo, I. Sasaki, Y. Kimoto, N. Hanzawa, Y. Teshima, T. Namba, Int. J. Cosmet. Sci., **28**, 299 (2006).
[6] J. W. Alexander, Surg. Infect. **10,** 289 (2010).
[7] S. H. Hsu, H. J. Tseng, Y. C. Lin, Biomaterials, **31**, 6796 (2010).
[8] J. Hasan, R. J. Crawford, E. P. Ivanova, Trends Biotechnol, **31**(5), 295 (2013).
[9] M. Spear, Plast. Surg. Nurs. **30**, 90 (2007).
[10] J. N. Israelachvili, Intermolecular Surface Forces, 2nd ed, Academic Press, San Diego (1992).

[11] A. Henglein, D. Meisel, J. Phys. Chem. **102**, 64 (1998).
[12] J. Fendler, Chem. Eng., Korean J. **18**, 1 (2001).
[13] A. Y. Peleg, D. C. Hooper. N. Engl. J. Med. **362**, 1804 (2010).
[14] V. D. Rosenthal, D. G. Maki, R. Salomao, C. A. Moreno, Y. Mehta, F. Higuera, L. E. Cuellar, O. A. Arikan, R. Abouqal, H. Leblebicioglu, Ann. Intern. Med. **145**, 582 (2006).
[15] Gaynes R., Edwards J. R, Clin. Infect. Dis. **41**, 848 (2005).
[16] P. T. Hammond, AIChE J. **57**, 2928 (2011).
[17] J. Lyklema, L. Deschenes, Adv Colloid Interface Sci. **168**(1–2), 135 (2011).
[18] J. Friedrich, Plasma Proces. Poly. **8**, 783 (2011).
[19] C. M. Simonescu, In Tech, **49**. 92 (2012).
[20] G. S. Deshmukh., S. U. Pathak, D. R. Peshwe, J. D. Ekhe, Bull. Mater. Sci., **33**(3), 277 (2010).
[21] B. Plav, S. Kobe1, B. Orel, Kovine, Zlitine, Tehnologije, **33**(6), 517 (1999).
[22] T. J. S. Learner, Analysis of Modern Paint, Gety Publication Amazom.com, (2005).
[23] M. C. M. Van Loosdrecht, J. Lyklema, W. Norde, G. Schraa, A. J. B. Zehnder, Appl. Environ Microbiol. **53**(8), 1893 (1987).

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