

# Silver Nanoparticles Used to Obtain Cellulosic Materials with Antibacterial Properties

OANA BORHAN<sup>1</sup>, AUGUSTIN MURESAN<sup>1\*</sup>, CEZAR DORU RADU<sup>1</sup>, EMIL MURESAN<sup>2</sup>, CRISTINA RIMBU<sup>3</sup>,  
IOAN GABRIEL SANDU<sup>4,5</sup>

<sup>1</sup> Gheorghe Asachi Technical University, Faculty of Textiles, Leather Engineering and Industrial Management, 29 Mangeron Blvd., 700050, Iasi, Romania

<sup>2</sup> Gheorghe Asachi Technical University, Faculty of Chemical Engineering and Environmental Protection, 73 Mangeron Blvd., 700050, Iasi, Romania

<sup>3</sup> Ion Ionescu de la Brad University of Agricultural Sciences and Veterinary Medicine Iași, Public Health Department, 3, Aleea Mihail Sadoveanu Str. 700490, Iasi, Romania;

<sup>4</sup> Gheorghe Asachi Technical University, Faculty of Materials Science and Engineering, 41 Mangeron Blvd., 700050, Iasi, Romania

<sup>5</sup> Romanian Inventors Forum, 3 Sf. P. Movila Str., L11, III/3, 700089, Iasi, Romania

*Cellulosic materials are ones of the most frequently used because they have the ability to absorb moisture, but under certain conditions of humidity and temperature they can be subjected to microbial attack. In this study, an attempt has been made to obtain antimicrobial finishing on cotton material by the using of silver nanoparticles (AgNPs) and poly(acrylic acid)(PAA). Silver nanoparticles, were obtained by reducing silver ions from AgNO<sub>3</sub> solution by various exposure methods to UV radiation. The surface characterization of materials was performed using scanning electron microscopy (SEM) and energy dispersive X-ray analysis (EDAX). The results indicated that AgNPs were successfully integrated and dispersed in the matrix. Antibacterial activity was evaluated against Gram negative bacteria (Escherichia coli) and Gram positive bacteria (Staphylococcus aureus). The application of antimicrobial treatment with poly(acrylic acid) and AgNPs on the material confers durability to washing.*

**Keywords:** cellulosic materials, poly(acrylic acid), UV activation, silver nanoparticles, antimicrobial activity

Antimicrobial finishes have been applied due to the development of microorganisms in the textile materials. The microbial infestation of textile materials represents a threat to the human life. The microorganisms located onto raw textile materials are growing during the wet processing, over the storage of finished products, during the transport and during the use of finished products. The unpleasant odors arising from the textile articles worned directly on the body, which are the effects of the growing of harmful microorganisms can spread diseases and affect the garments [1]. Nowadays, silver nanoparticles are used due to their antimicrobial effect and due to their wide range of applications in different scientific researches and industries.

Generally, the antimicrobial mechanism of the chemical agents is related to the specific binding with surface and to the metabolism of agents into the microorganism. Various microorganisms have evolved drug resistance over many generations. Thus far, these antimicrobial agents based on chemicals have been effective for therapy; however, they have been limited to use for medical devices and in prophylaxis of antimicrobial facilities. Therefore, an alternative way to overcome the drug resistance of various microorganisms is needed desperately, especially in medical devices etc.

The toxicity of silver ion and its compounds has been confirmed for bacteria and microbes; therefore, they are used in the producing of wound dressings and can be applied for the manufacture of antibacterial food packaging materials [2-3]. Silver is a more secure antibacterial agent compared to other organic antibacterials. The last ones

have been constantly avoided because of their harmful effects on the human body [4-12].

The silver salts are very soluble in water and have been used as antiseptic agents. Species of silver ions can also be obtained by ion exchange using complexes of silver with other inorganic materials (for example, complexes of silver-zeolite [13, 14]). For the silver to gain antimicrobial effect, free water must be present. Depending on the source of silver ion, ion exchange processes have a delayed activity compared with those derived from the water-soluble salts. The activity seems to increase with the increase of the temperature and pH.

Previous studies have proved that the silver nanoparticles give toxicity to microorganisms such as *Bacillus subtilis* and *Klebsiella mobilis* [15], *Staphylococcus aureus* and *Escherichia coli* [16], *Pseudomonas aeruginosa* and *Klebsiella pneumonia* [17], *Streptococcus pyogenes* and *Salmonella typhi* [18]. It is considered that the silver nanoparticles can act as antimicrobial agent by means of two mechanisms interfered in respiratory metabolism of the organism.

One of the accepted mechanisms about the antimicrobial properties of silver ions is related to their ability to penetrate into the cell wall and to react with the -SH groups of enzymes leading to the alteration of the major cell metabolisms and inhibiting the growth of bacteria [19, 20]. The formation of free radicals on the surface of the silver nanoparticles is considered to be another mechanism through which the cells are destroyed. These free radicals have the ability to damage the cells walls which finally lead to the death of the cell [21, 22]. Applying of AgNPs on

\* email: aug\_muresan@yahoo.com

textile fabrics has significantly grown in the last years [23, 24].

Silver nanoparticles (AgNPs) are among the nanomaterials that can be encountered in the daily life, most often as result of antimicrobial finishing of the textiles [25-27].

The properties of the textile materials can be further improved in terms of performance as well as durability in order to increase the degree of comfort and hygiene which makes them comfortable to wear.

## Experimental part

### Materials and methods

In this study we used cellulosic materials obtained from cotton fabrics 100% (141 g/m<sup>2</sup>) which have previously been alkali treated and then bleached. For the synthesis of AgNPs, silver nitrate (99.9%, Sigma-Aldrich), poly(acrylic acid) (PAA) (M.W. 100,000); 35% (w/w) aqueous solution, Sigma Aldrich, were used without further purification.

### Obtaining of the silver nanoparticles

The silver nanoparticles were prepared by the reduction of silver ions from the silver nitrate solution under the action of UV radiation and poly(acrylic acid) (fig. 1).

The solution was poured in a Petri dish and then to a UV lamp with the main emission band from 200 to 300 nm (the distance from UV lamp to the Petri dish was 10 cm). The required amount of PAA solution was added before the addition of the AgNO<sub>3</sub> solution in order to achieve a final PAA concentration of  $1.3 \times 10^{-7}$  mol/L. All the AgNPs syntheses were performed at the room temperature (25°C), under vigorous stirring. The concentration of AgNO<sub>3</sub> used in experiments was varied between  $1 \times 10^{-6}$  -  $4 \times 10^{-6}$  mol/L ( $1.7 \times 10^{-4}$  g/L -  $8.5 \times 10^{-4}$  g/L). The reaction times were ranged from 0 to 10 min in order to achieve the complete conversion.

The absorption spectrum of the colloidal product was recorded at different times and concentrations using a UV-Visible Camspec M501 Single Beam Canning Spectrophotometer.

### AgNPs application on cotton fabric

AgNPs obtained was brought in the PAA concentrated solutions (5 and 7%), homogenized, after which they were applied to the fabric (fig.1).

In the first stage of the treatment, the fabric samples were padded (90% wet pick up) with the prepared emulsion and dried for 20 min at 80°C. Finally these were subjected to the heat treatment at 140°C for 3 min. The samples thus obtained have been the subject of the subsequent analyses.

### FTIR-ATR Analysis.

FTIR analyses were carried out on a multiple internal reflectance accessory (SPECAC, SUA) with an ATR KRS-5 crystal of thallium bromide iodide, having 25 reflections and the investigation angle of 45°. This accessory device was attached to the FTIR spectrophotometer IRAffinity-1 Shimadzu (Japan), the spectra registration was performed with 250 scans in the 1800-600 cm<sup>-1</sup> range. After registration, the absorption spectra have been electronically superposed (using the Panorama soft from LabCognition Co.).

### SEM-EDAX analysis of the treated cotton samples

A QUANTA 200 3DDUAL BEAM electron microscope was used, which is a combination of two systems (SEM and focused ion beam (FIB)), by whose means, by sending an

electron beam on the treated samples, three-dimensional images could be obtained, with a magnification of 100000X. Moreover, by the use of the energy-dispersive X-ray analysis (EDAX), elemental analyses were possible for the identification of the surface characteristics and a high resolution chemical analysis.

### Antimicrobial testing

The microbiologic investigations have been performed at Microbiology-Immunology Laboratory of the University Centre of Medical and Veterinary Researches, within the Faculty of Veterinary Medicine of Iasi, Romania.

Accordingly, the antimicrobial evaluation was carried out with respect to the standardized stems of *Staphylococcus aureus* ATCC 29213 and *Escherichia coli* ATCC 25922, both stems being recommended by the Clinical and Laboratory Standards Institute (CLSI, previously NCCLS) for the method of diffusion in agar. With this aim in view, young cultures of 24 h obtained in the liquid culture medium (nutritive broth). The bacterial inoculus was brought at the standard density of 0.5 McFarland at which, for most of the bacterial species, 1 mL solution contains  $(1 \div 2) \times 10^8$  cfu/mL (colony forming units).

Muller Hinton agar (Oxoid) was used as nutritive solid medium, because it possesses a nutritive value that permits the optimal development of a large variety of germs and does not contain inhibitors of some antimicrobial substances. After melting and cooling down to 45°C, samples of 9 mL were distributed on sterile Petri dishes in which had been previously deposited by 1 mL of tested bacterial culture from the dilution tube.

From the textile materials treated with AgNPs and poly(acrylic acid) were taken with side of 1 cm (like in the disk diffusion method). They were cut in aseptic conditions in the bacteriological vapor hood with laminar flow, in order to avoid additional contamination of the samples, which could corrupt the results.

The samples were disposed circularly at equal distances on the surface of a solidified Muller Hinton medium. The plates were incubated in a thermostat at 37°C, the results being interpreted after 24 hours.

## Results and discussions

### Chemical Mechanisms

The mechanism for the formation of Ag nanoparticles under UV irradiation in aqueous solutions of PAA is based on the reduction of Ag<sup>+</sup> by reactive species from the photochemical reaction of PAA. Initially, the solution of AgNO<sub>3</sub> and PAA was colorless, but, following UV irradiation with increasing time, obvious color changes from colorless to light yellow, then to brown, and finally to dark brown were observed (fig.1).

As is seen in figure 2, before the irradiation, the solution showed no absorption in the wavelength range from 350 to 800 nm.

After 3 min of UV irradiation, a weak absorption band centered at about 430 nm appeared. By further extending of the irradiation time from 3 to 5 min, the intensity of this absorption band increased significantly. At the same time, a peak at 430 nm was also observed upon the irradiation. The increase in the absorbance of the absorption peak indicated the gradual growth of Ag nanoparticles upon prolonged UV irradiation. In the absorption spectra, an increase of the absorbance with the increasing of the AgNO<sub>3</sub> concentration was noticed. The peak at 430-460 nm is assumed to occur due to the interactions of PAA with Ag<sup>+</sup> ions and Ag nanoparticles as revealed in figure 3.

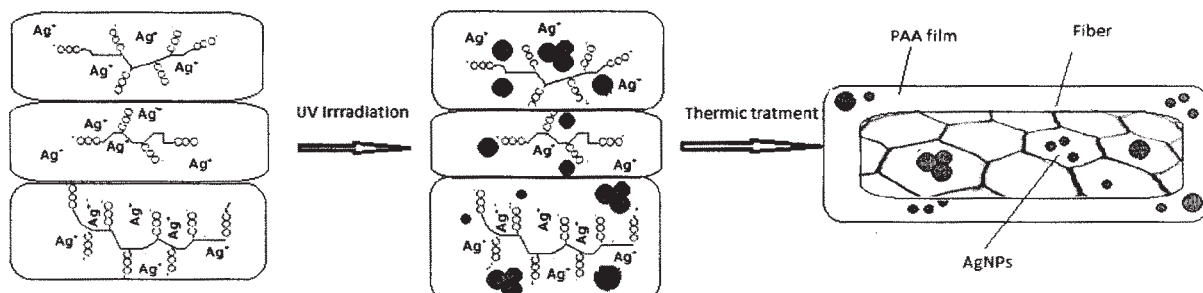


Fig.1. Preparation of the silver nanoparticles with UV lamp and their fixing on cotton

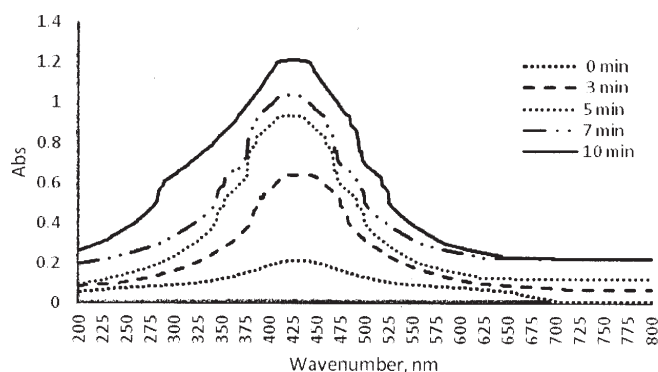


Fig. 2. Absorption spectra of silver nanoparticle colloids produced from a  $\text{AgNO}_3$  solution with a concentration of silver of  $[\text{Ag}^+] = 3 \times 10^{-6} \text{ mol/L}$  (0.00051 g/L) by UV irradiation under variation of exposure time

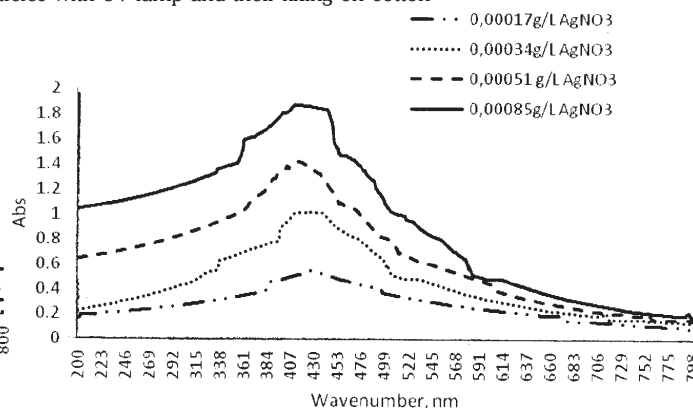


Fig. 3. Absorption spectra of the silver nanoparticle colloids produced from  $\text{AgNO}_3$  solution with various concentrations of silver ions of  $[\text{Ag}^+]$  ( $1.7 \times 10^{-4} \text{ g/L}$  –  $8.5 \times 10^{-4} \text{ g/L}$ ) by UV irradiation for 7 min

#### FTIR-ATR analysis of treated cotton samples with AgNPs and poly(acrylic acid)(PAA)

After registration, the absorption spectra have been superposed using OPUS software and are shown in figure 4.

By the comparing of the spectra corresponding to the untreated cotton and respectively polyacrylic acid treated cotton one notices that the differences occur only in the  $2850\text{--}3000 \text{ cm}^{-1}$ , and  $1470\text{--}1450 \text{ cm}^{-1}$  ranges and around the  $1700 \text{ cm}^{-1}$  value.

The differences noticed in the  $3000\text{--}2850 \text{ cm}^{-1}$  range are attributed to the stretchings vibrations of C-H bonds from polyacrylic acid, rich in methylene and methyldene groups. The increasing of the number of C-C bonds is also confirmed by the increasing of the absorption band in the range  $1470\text{--}1450 \text{ cm}^{-1}$  assigned to the C-H bond. The occurrence of the peaks at  $1728 \text{ cm}^{-1}$  (for C = O stretching vibration) and the increase of the peak intensity from  $1300 \text{ cm}^{-1}$  corresponding to the C-O bond stretching vibration confirms the presence of carboxylic group originating from the polyacrylic acid.

Antimicrobial effects of durability has been tested by repeated washings and were determined in agreement with the standard SR EN ISO 105-C06:1999, the treated samples were subject from one to four repeated washings.

The presence of Ag on the surface of the fabric treated with poly(acrylic) acid is proved by the decrease of the FT-IR ATR absorption bands in the range  $3000\text{--}2800 \text{ cm}^{-1}$  and by the increases of the peaks intensities at  $1354.5 \text{ cm}^{-1}$  and  $804.91 \text{ cm}^{-1}$  as can be noticed from the spectra 1-3 (fig. 5).

This assumption resulted also from the comparison of the FTIR ATR spectra spectra performed by the transmission method for the films formed only from acrylic acid and Ag. It is likely that the UV radiation reduces the silver ions leading to the lowering of the peaks at  $2959.4 \text{ cm}^{-1}$ . Thus is explained the decrease occurred in the range  $3000\text{--}2950 \text{ cm}^{-1}$ . The washing durability is good, just after 4 washings a part of the Ag is removed which lead to the increase of the peak from this area.

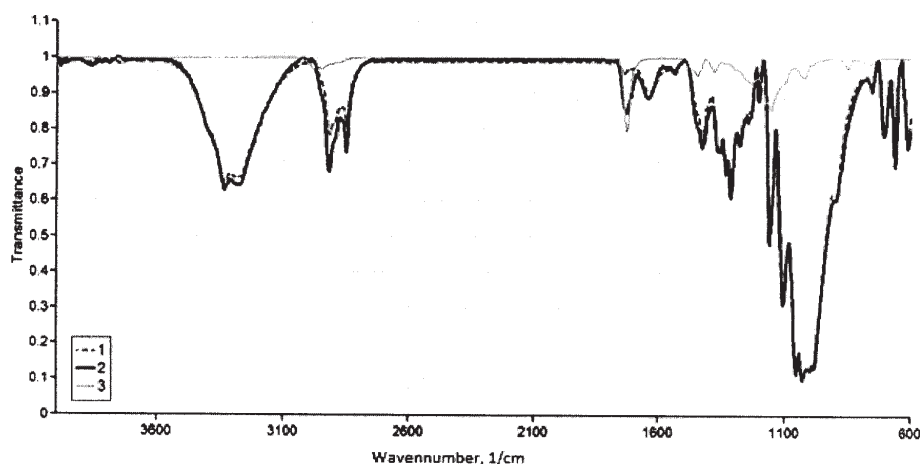


Fig. 4. Spectra FTIR-ATR:  
1-Cotton untreated; 2- Cotton treated with AgNps and poly(acrylic) acid;  
3- poly(acrylic) acid



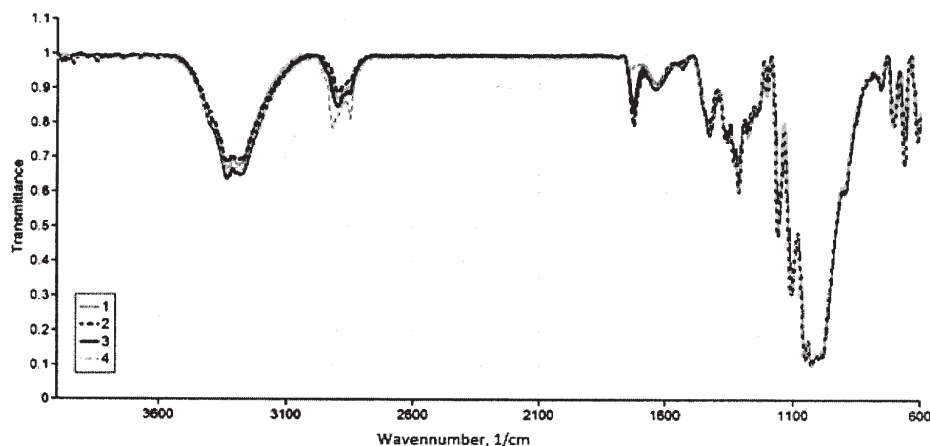


Fig. 5. Spectra FTIR-ATR  
1 - treated cotton; 2 - treated cotton after the first washing;  
3 - treated cotton after the fourth washing; 4 - untreated cotton

#### SEM and EDAX results for the cotton samples treated with AgNPs

From these images it is to be noted that on the surface of fibers was fixed polymer PAA which has packed and silver ions in the fleet of dealing with exposed UV. EDAX analysis shows that in addition to fixing the polymer is also fixing the silver ions on the fabric. As a result of these analyzes it can be established that the durability treatment achieved by this method is good. The presence of silver on cotton samples is confirmed by the SEM images (fig. 6) and EDAX results. EDAX analysis led to a series of quantitative informations concerning the Ag content in the cotton samples, after the treatment. The AgNPs content decreases by increasing the number of wash cycles.

Therefore, at the end of the 4<sup>th</sup> laundering cycles there is enough silver to confer the samples an antimicrobial effect.

So the content of silver ion is maintained on fabric and after 4 washes them ranging from 2.87 % to 4.25 %, for the treatments in which we used 5% polyacrylic acyl. These results are confirmed by antimicrobial tests.

#### Antimicrobial activity

The antimicrobial activity of the cotton samples treated with AgNPs and poly(acrylic acid) was analyzed against two well-known bacteria: *Escherichia coli* (a gram-negative bacteria) which is one of the most common microorganisms that can be selected for the antimicrobial tests and is resistant to the common antimicrobial agents, as well as the *Staphylococcus aureus* (a gram-positive bacteria) which is the major cause of diseases in hospitals [28, 29].

The circular samples were cut to a 10 mm diameter. After the incubation period, the diameter of the inhibition zone was determined for each sample. The visual aspects of the antibacterial tests are presented in figure 7 and the inhibition diameters are compared in table 1. The results in Table 1 show that most of the treated samples presented an antibacterial effect. The best results were obtained against the *Escherichia coli* bacteria.

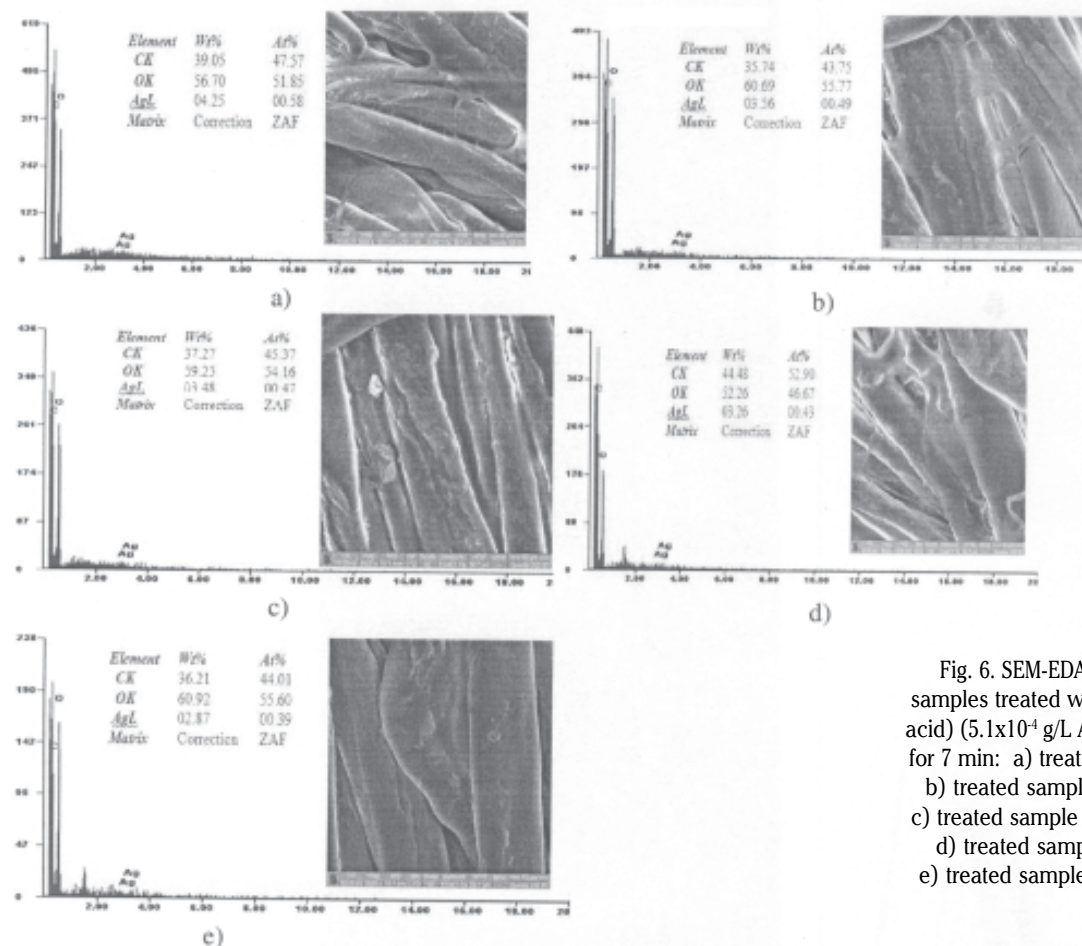


Fig. 6. SEM-EDAX images of the cotton samples treated with AgNPs and poly(acrylic acid) ( $5.1 \times 10^{-4}$  g/L  $\text{AgNO}_3$ ) exposed to UV light for 7 min: a) treated sample and unwashed, b) treated sample after the first washing, c) treated sample after the second washing, d) treated sample after the third wash, e) treated sample after the fourth washing

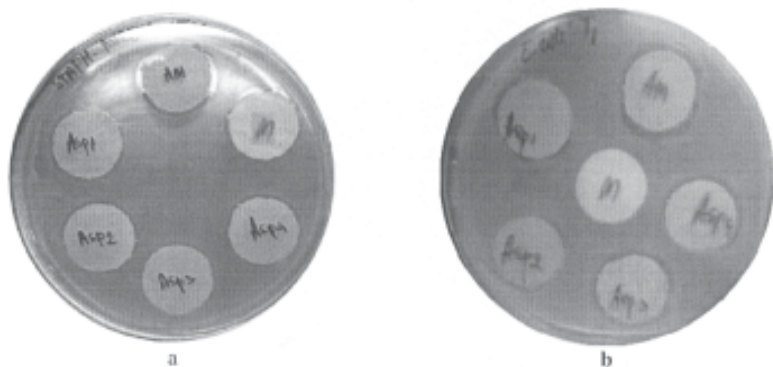


Fig. 7. Visual aspects of the cultures after incubation with *Staphylococcus aureus* ATCC-29213 (a) and *Escherichia coli* ATCC 25922 (b):

M- untreated sample, AM- sample treated with AgNPs and PAA ;  
Asp1- sample treated after the first washing; Asp2 - treated sample after the second washing; Asp3- treated sample after the third washing; Asp4- treated sample after the fourth washing

Sample	Diameter of the inhibition zone (mm)	
	<i>Staphylococcus aureus</i> ATCC-29213	<i>Escherichia coli</i> ATCC 25922
Sample of untreated cotton	-	-
Sample of cotton treated and unwashed	11.5	14.6
treated cotton sample after the first washing	11.2	12.3
treated cotton sample after the second washing	11.1	11.9
treated cotton sample after the third washing	10.7	11.9
treated cotton sample after the fourth washing	10.7	11.9

**Table 1**  
ANTIMICROBIAL ACTIVITY

The highest changes of the inhibition zone diameters were obtained against bacteria *Escherichia coli*. The values of the inhibition zone diameters against *Escherichia coli* decreased from 14.6 mm for the sample treated and unwashed to 11.9 for the washed sample, while the values of the inhibition zone diameters corresponding to *Staphylococcus aureus* decreased from 11.5 to 10.7 cm.

#### Durability of the treatment

The durability effect of the treatment with AgNPs was determined with the home laundering test. According to the standard test SR EN ISO 105-CO6:1999, the samples were subjected to 4 repeated home laundering cycles. The tests were carried out with a Mathies Polycolor 2002 machine from Bezema Co., followed by rinsing with distilled water at 40°C and then by drying at the room temperature. The durability of the treatment with AgNPs has been assessed by measuring the Ag content remained on the textile materials, using the EDAX option of the electronic microscope.

The results of the tests have shown the antimicrobial activity of the fabric treated with poly(acrylic acid) and AgNPs even after the four washes but the control sample test antimicrobial effect is absent. It was found a differential of the two micro-organisms from fabric treated which might be correlated with different composition and ultrastructure of the cell wall to the two species.

In all the cases the diameter of the inhibition zone decreases with the increase of the samples washing number. This parameter is also correlated with the silver content onto the surface of the samples.

#### Conclusions

From this work the following conclusions can be drawn:  
- AgNPs can be obtained by reduction of the silver ions from  $\text{AgNO}_3$  solutions under the influence of UV radiations and in the presence of poly(acrylic acid);

- the amount of AgNPs is influenced by the concentration of the used  $\text{AgNO}_3$  solution and by the UV irradiation time;

The silver particles could be deposited on a textile fabric by applying the colloidal solution in a simple finishing process.

- The results of the application of antimicrobial treatment with AgNPs and poly(acrylic) acid show that the content of silver ions existing on the fabric is an indicator for the durability of the washes.

- The content of AgNPs onto the textile fabric was evidenced by FTIR spectroscopy and SEM-EDAX analyses. By the using of polyacrylic acid the durability of the treatment is increased to repeated washings which indicates the effectiveness of the antibacterial treatment carried out.

#### References

- MUCHA, H., HOTER, D., SWEREW, M., Melland International, **8**, 2002, p. 148.
- MANEERUNG, T., TOKURA, S., RUJIRAVANIT, R., Carbohydr. Polym. **72**, 2008, p. 43.
- IP, M., LUI, S.L., POON, V.K.M., LUNG, I., BURD, A., J. Med. Microbiol. **55**, 2006, p. 59.
- INOUE, Y., HOSHINO, M., TAKAHASHI, H., NOGUCHI, T., MURATA, T., KANZAKI, Y., HAMASHIMA, H., SASATSU, M., J. Inorg. Biochem. **92**, 2002, p. 37.
- DASTJERDI, R., MONTAZER, M., SHAHSAVAN, S., Colloids Surf., **B** **81**, 2010, p. 32.
- TEODORESCU, D., HODOSAN, C., NISTOR, L., HUBCA, G., Mat. Plast., **51**, no. 2, 2014, p. 133.
- TURA, V., TOFOLEANU, F., MANGALAGIU, I., MINDRU, T.B., BRINZA, F., SULITANU, N., SANDU, I., RAILEANU, D., IONESCU, C., Journal of Optoelectronics and Advanced Materials, **10**, no. 12, 2008, p. 3505.
- TEODORESCU, D., HODOSAN, C., NISTOR, L., HUBCA, G., Mat. Plast., **51**, no. 3, 2014, p. 296.
- TOFOLEANU, F., MINDRU, T.B., BRINZA, F., SULITANU, N., SANDU, I.G., RAILEANU, D., FLORISTEAN, V., HAGIU, B., IONESCU, C., SANDU, I., TURA, V., Journal of Optoelectronics and Advanced Materials, **10**, no. 12, 2008, p. 3512.
- CRACIUNESCU, M.C., NEGRUTIU, M.L., HOGEA, E., FREIMAN, P.C., BOARIU, M., CRACIUNESCU, E., SINESCU, C., Mat. Plast., **51**, no. 4, 2014, p. 414.
- COPCIA, V.E., GRADINARU, R., MIHAI, G.D., BILBA, N., SANDU, I., Rev. Chim. (Bucharest), **63**, no. 11, 2012, p. 1124.
- POPESCU, V., MURESAN, A., CONSTANDACHE, O., LISA, G., MURESAN, E.I., MUNTEANU, C., SANDU, I., Industrial & Engineering Chemistry Research, **53**, no. 43, 2014, p. 16652.

13. WEBB G.F, D'AGATA E.M, MAGAL P., RUAN S., Proc. Natl. Acad. Sci. USA, **102**, 2005, p. 13343.
14. GALEANO, B., KORFF, E., NICHOLSON, W. L., Microbiology., **69**, no. 7, p. 4329.
15. ZHANG, Y., PENG, H., HUANG, W., ZHOU, Y., YAN, D., Journal of Colloid and Interface Science, **325**, no. 2, 2008, p. 371.
16. KYUNG, H.C., PARK, J.E., OSAKA, T. PARK, S.G., Electrochimica Acta, **51**, no. 5, 2005, p. 956.
17. WON, K.S., YOUK, H.J., PARK. W.H., Carbohydrate Polymers, **6**, no. 5, 2006, p. 430.
18. ANIMA, N., SARAVANAN, M., Nanomedicine: Nanotechnology, Biology, and Medicine, **5** no. 4, 2009, p. 452.
19. MORONES, J.R., ELECHIGUERRA, J.L., CAMACHO, A., HOLT, K., KOURI, J.B., RAMIREZ, JT, YACAMAN, MJ, Nanotechnology, **16**, 2005, p. 2346.
20. HATCHETT, D.W., HENRY, S. J, Phys. Chem., **100**, 1996, p. 9854.
21. DANILCAUK, M., LUND, A., SALDO, J., YAMADA, H., MICHALIK, J., Spectrochimica. Acta. Part A., **63**, 2006, p. 189.
22. KIM, J.S., KUK, E., YU, K., KIM, J.H., PARK, S.J., LEE, H.J., KIM, S.H., PARK, Y.K., PARK, Y.H., HWANG, C.Y., KIM, Y.K., LEE, Y.S., JEONG, D.H., CHO, M.H., Nanomedicine, **3**, 2007, p.95.
23. BRAGG, P.D., RAINNIE, D.J., Can. J. Microbiol., **20**, 1973, p. 883.
24. CHEN, C.Y., CHIANG, C.L., Mater. Lett. **62**, 2008, p. 3607.
25. EL-RAFIE, M.H., MOHAMED, A.A., SHAHEEN, TH.I., HEBEISH, A., Carbohydr. Polym. **80**, 2010, p. 779.
26. VIRENDER, K., SHARM, RIA A. YNGARD, YEKATERINA LIN, Advances in Colloid and Interface Science, **145**, 2009, p.83.
27. LEE, H.J., YEO, S.Y., JEONG, S.H., J. Mater. Sci. **38**, 2003, p. 2199.
28. HAN, S., YANG, Y., Dyes Pigm. **64**, 2005, p. 157.
29. ABRAMIUC, D., CIOBANU L., MURESAN, R., CHIOSAC, M., MURESAN, A., Fibers and Polymers, **14**, 2013, No.11, p. 1826.

---

Manuscript received: 18.01.2015