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Antimicrobial Effectiveness of Cellulose based Fabrics treated with Silver Nitrate Solution using Plasma Processes

Protimikrobna učinkovitost celuloznih tkanin, obdelanih z raztopino srebrovega nitrata v plazmi

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Abstract

In order to obtain antibacterial properties, the possibility of deposition of silver particles from silver nitrate ($AgNO_3$) solutions by plasma deposition process using argon as a carrier gas (PDP-Ar) was explored. Hexamethyldisiloxane and acrylic acid were used as precursors and were deposited by plasma enhanced-chemical vapor deposition (PE-CVD). The processes were carried out on lyocell and modal fabrics and antimicrobial efficacy was determined on *E. coli* and *S. aureus* using time kill assay method. The results of minimal inhibitory concentration (MIC) show that higher antimicrobial efficacy on *E. coli* is exhibited by the solution of ($AgNO_3$) in ethylene-glycol (0.066 µg/ml) rather than in absolute ethanol (0.265 µg/ml). For *S. aureus*, minimal inhibitory concentrations of $AgNO_3$ solutions in both absolute ethanol and ethylene-glycol as solvents are obtained at the same value (0.132 µg/ml). Overall, the best antibacterial effect for both modal and lyocell samples has been achieved against *E. coli* using treatments with precursors (AAC and HMDSO) and $AgNO_3$ in ethylene-glycol as solvent, with prolonged incubation time.
Keywords: cellulose fabrics, plasma processes, $AgNO_3$, quantitative microbiological method, antibacterial efficacy

Izvleček

Za doseganje protibakterijskih lastnosti je bila raziskana možnost nanosa srebrovih delcev iz raztopin srebrovega nitrata ($AgNO_3$) v plazmi, kjer je bil kot nosilni plin uporabljen argon (PDP-Ar). Kot prekurzorja sta bila uporabljena heksametildisiloksan in akrilna kislina, ki sta bila nanesena iz parne faze v plazmi (PE-CVD). Postopki so bili izvedeni na tkaninah iz liocelnih in modalnih vlaken, protimikrobna učinkovitost pa je bila določena na E. coli in S. aureus z metodo določanja preživelosti. Rezultati minimalne inhibitorne koncentracije (MIC) kažejo, da ima raztopina srebrovega nitrata v etilenglikolu (0,066 µg/ml) višjo protimikrobno učinkovitost na E. coli kot v absolutnem etanolu 0,265 µg/ml). Za S. aureus so bile MIC $AgNO_3$ raztopin v obeh topilih, absolutnem etanolu in etilenglikolu, dosežene pri enaki vrednosti (0,132 µg/ml). Na splošno je bil najboljši protibakterijski učinek proti E. coli s podaljšanim inkubacijskim časom dosežen za vzorce iz modalnih in liocelnih vlaken, obdelanih s prekurzorji (AAC in HMDSO) in $AgNO_3$ v etilenglikolu kot topilu.
Ključne besede: celulozne tekstilije, $AgNO_3$, kvantitativna mikrobiološka metoda, protibakterijska učinkovitost

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1 Introduction

Plasma technology and its application in the field of textile technology are known as ecologically friendly processes. Textile materials exposed to plasma undergo through chemical and physical transformations in the surface layer. Using different gasses and reagents in the process of plasma finishing of textile materials, a variety of functional properties can be obtained. Those include flame resistance, antistatic, hydrophobic, antibacterial properties etc. The ultimate effect depends on many more factors such as plasma type, type of materials, plasma parameters, treatment conditions and the process used [1, 2].

In this study, the application of low-pressure plasma for the enhancement of surface properties of lyocell and modal fibers and the achievement of antibacterial properties by deposition of silver particles was explored. Natural materials are an excellent media for the growth of microorganisms, especially in humid and warm conditions. While many synthetic fibers have good resistance against microbial attack, natural fibers are more easily affected. Proteins in keratin fibers and carbohydrates in cellulose fibers can serve as a source of food and energy for various bacteria, fungi and molds [3]. Bacteria are single-celled organisms that reproduce very quickly in appropriate conditions. They are classified as gram-positive and gram-negative and certain types are pathogenic and might cause serious health problems [4]. Antimicrobial treatments are carried out in order to control the growth and reproduction of microorganisms. Agents can kill bacteria or fungi (biocides) or control the growth and spread of microbes (biostatics). Both prevent the spread of infection, allergic and respiratory problems as well as degradation of textile materials in the form of discolouration, staining, odors and degradation of the fibers. There are various antimicrobial agents used which differ in their chemical composition and antimicrobial activity. Quaternary ammonium compounds, polyhexamethylene biguanide, triclosan and some heavy metals can be used for biocidal purposes whereas chitosan and plant-derived bioactive agents can be used as biostatics [5]. Many heavy metals such as silver, copper, zinc and cobalt have excellent antimicrobial properties at very low concentrations but silver is the most commonly used in the field of textile industry [6]. It can be used as a metal, salt and nanoparticle [7]. Single mechanism of antimicrobial action is not fully understood, but some mechanisms have been proposed that suggest that silver ion reacts with DNA and RNA molecules within the cell wall of microorganisms and prevents the replication. In addition, a reaction of silver with thiol groups (–SH) inside the cell is assumed which causes protein deactivation and reduction of enzyme activity. This causes changes in metabolism, prevents propagation and ultimately leads to the destruction of microorganism [8–10]. Also, it is proposed that silver acts by binding to key functional groups of enzymes. Bacterial plasma or cytoplasmic membrane are an important target site for silver ion, which causes the release of K^+ ions from bacteria. Silver ions inhibit cell division and damage the cell envelope and content of bacteria [11]. Antimicrobial textiles are used in medical technology, health, hygiene products, home textiles, water treatment systems, as well as clothes for everyday use and personal protection [4, 12].

Physical sputtering and Plasma Enhanced Chemical Vapor Deposition (PE-CVD) under low-pressure plasmas are dominant techniques used in textile finishing [1]. In this study, a relatively new method was used for the deposition of antibacterial agent called Direct Plasma Deposition Process (PDP) (Figure 1).

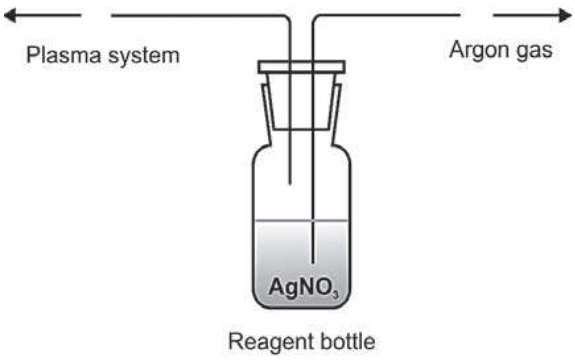

Figure 1: Reagent bottle for direct plasma deposition process

The process consists of reagent bottle with antibacterial agent, which is from one side connected to the supply of argon gas and the other side to plasma system. Argon is used as a carrier gas, which assists the transfer of reagent to the plasma system and on the textile substrate.

2 Materials and methods

2.1 Textile samples and chemical agents

Plain wave lyocell (CLY) and modal (CMD) fabrics (Lenzing, Austria) were used in this study. They were industrially prepared (desized and scoured) and weaved at the textile company Čateks, Croatia.

By dissolving $AgNO_3$ (Sigma Aldrich) in absolute ethanol (Carlo Erba) and ethylene-glycol (Fluka Analytical), 0.1 M solutions were prepared and used for processing the fabrics in order to achieve antibacterial properties. Hexamethyldisiloxane, HMDSO (Sigma Aldrich, ≥98.5%) and acrylic acid, AAC (Acros Organic, 99.5%) were used as precursors. Applied bacterial species were *Escherichia coli* ATCC 10536 (gram-negative bacteria) and *Staphyloccocus aureus* ATCC 6538 (gram-positive bacteria). The strains were grown in Tryptic soy agar (105458 Tryptic soy agar, Merck Millipore, Germany). Müeller-Hinton Broth (BBLTM Müeller-Hinton Broth – BD, USA) was used as a nutrient broth medium for broth microdilution technique.

2.2 Plasma treatments

Treatments were done using low-pressure plasma system type NANO LF-40 kHz, by Diener. To ensure enhanced binding of silver particles on textile surface, activation process was carried out for 5 minutes using O_2 gas (purity of 99.99%, Messer) under optimized process parameters – pressure 0.34 mbar, power 300 W, frequency 40 kHz and gas flow 40 cm^3/min. The pretreatments with HMDSO and AAC were conducted in order to enhance adsorption of silver particles onto cellulose surface using PE-CVD process. The process was conducted for 20 minutes at pressure of 0.18 mbar and power of 150 W. $AgNO_3$ solutions were applied on activated and pretreated samples using PDP-Ar process and argon plasma as a carrier gas for deposition. The process was carried out for 20 minutes at pressure 1.5 mbar, gas flow 40 cm^3/min and power 150 W.

2.3 FE-Scanning electron microscopy analysis

Field emission scanning electron microscopy (FE-SEM) was conducted on untreated and plasma treated samples using Tescan SEM microscope (Mira 3 LMU). Before testing, the samples were steamed by vaporized mixture of gold and palladium under argon plasma. Micrographs were obtained using 4kx, 7kx and 10kx magnifications.

2.4 Antimicrobial efficacy of silver nitrate solutions

Antimicrobial efficacy of $AgNO_3$ solutions was determined by known broth microdilution technique, which specifies minimum inhibitory concentration (MIC), i.e. the minimum concentration of antimicrobial agent that inhibits the growth of a microorganism after a specific time of incubation. The method is based on the dilution of the antimicrobial agent in the culture medium in microtiter plates, followed by addition of inoculum. If there is no growth of microorganism colonies, the concentration is the well represents MIC. For this purpose, a series of 12 concentrations of $AgNO_3$ was prepared from 4146.75×10^{-3} to 2.07×10^{-3} µg/ml. MICs were determined for *E. coli* and *S. aureus* after incubation at 37 °C for 24 hours using 2,3,5- triphenyltetrazolium chloride (TTC) as a redox indicator for determination of bacteria viability. In the presence of bacteria, TTC was reduced to red coloured substance triphenyl formazan (TPF) and the colour change was directly proportional to the viable active cells. The change in colour was measured spectrophotometrically at 540 nm (Labsystems iEMS MF, type 1404, filter F6).

2.5 Antimicrobial efficacy of treated samples

Antibacterial efficacy of treated materials was determined by time kill assay method. It is a quantitative microbiological method that determines the number of colony forming units of microorganisms after a specific time of incubation of samples. In this way, the rate at which a microorganism is killed as a function of time can be established. Treated samples (1cm x 1cm) were inoculated with 50 µl of microorganism suspension in sterile Petri dishes. The antibacterial effectiveness against *E. coli* and *S. aureus* was investigated at the moment of contact of the sample with a suspension of microorganisms (t_0) and after incubation time of 6 hours (t_1) and 18 hours (t_2) at 37 °C. Using sterile tweezers, inoculated samples were then transferred in test tubes, which had been pre-filled with 1 ml of saline. After mixing with vibromixer (Vortex vibromixer Genius 3, Ika, Germany) for 30 seconds, 100 µl of the solution was transferred to an Eppendorf tube of 2 ml that had been previously filled with 900 ml of saline solution. In this way, the solution was diluted 10 times. This solution was then serially diluted and the aliquots of the dilutions series were spread-plated on an agar medium to allow for enumerating the

colonies of the bacteria. Tryptic soy agar (105458 Tryptic soy agar, Merck Millipore, Germany) was used as a nutrient medium and quantification of the number of colony forming units of microorganisms was done after incubation at 37 °C in the dark for 24 hours. The process is shown schematically in Figure 2.

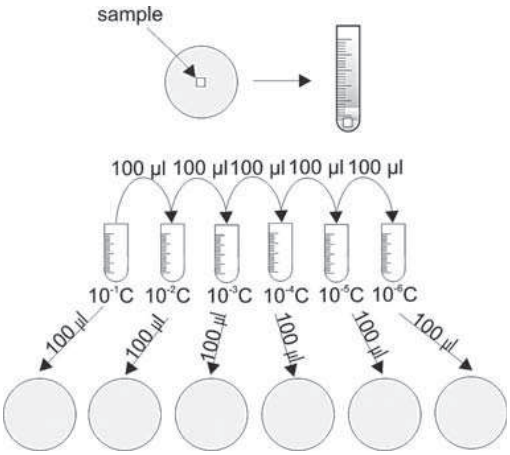

Figure 2: Schematic representation of time kill assay method

For every concentration and time point, colonies of viable bacteria were counted and CFU/ml (colony forming units per ml) was calculated according to Equation 1:

$$\frac{CFU}{ml} = \log_{10} \frac{\frac{no.\ of\ colonies}{volume\ of\ culture\ plate}}{dilution\ factor} \quad (1)$$

The final results are expressed as a percentage of inhibition in defined time intervals.

3 Results and discussion

3.1 Morfological characteristics of plasma treated samples

Figure 3 shows the obtained SEM micrographs of untreated and plasma treated lyocell and modal samples.

The SEM micrographs of untreated lyocell and modal samples show smooth, clear surface of fibers.

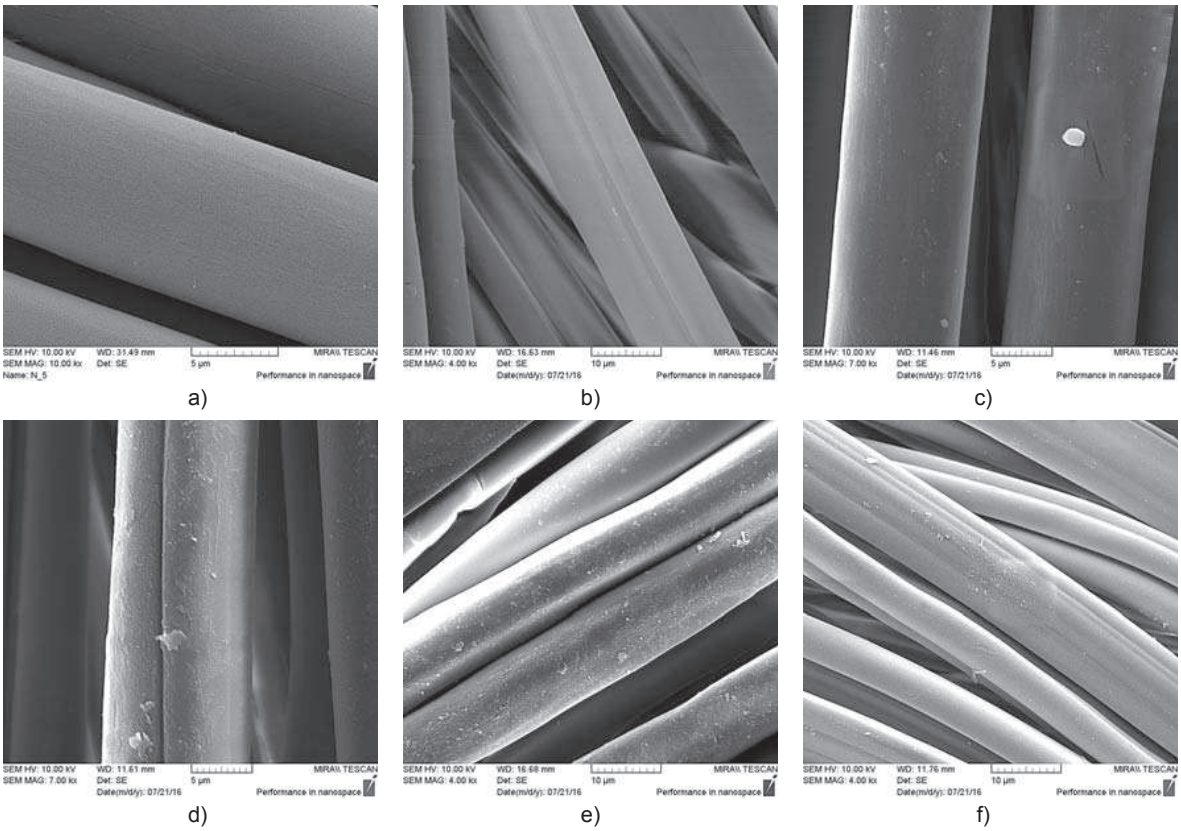

Figure 3: SEM micrographs of (a) untreated CLY and (b) CMD samples; (c) CLY treated with AAC and $AgNO_3$ solution in ethylene-glycol; (d) CMD treated with AAC and $AgNO_3$ solution in ethanol; (e) CLY treated with HMDSO and $AgNO_3$ solution in ethylene-glycol; (f) CMD treated with HMDSO and $AgNO_3$ solution in ethanol

On the surface of fibers treated with precursors (AAC, HMDSO) and $AgNO_3$ solutions, significant presence of precursors and silver particles over the entire fiber surface is evident which was deposited after surface activation with O_2 plasma. It confirms the effectiveness of treatments performed using plasma processes.

3.2 Minimal inhibitory concentrations of silver nitrate solutions

Antimicrobial effectiveness of silver nitrate on the bacterial species *E. coli* and *S. aureus* was determined. MIC values are presented graphically in Figure 4 and Figure 5. The value of the absorbance, which indicates the activity of microorganisms, in relation to a series of concentrations of tested solutions are shown.

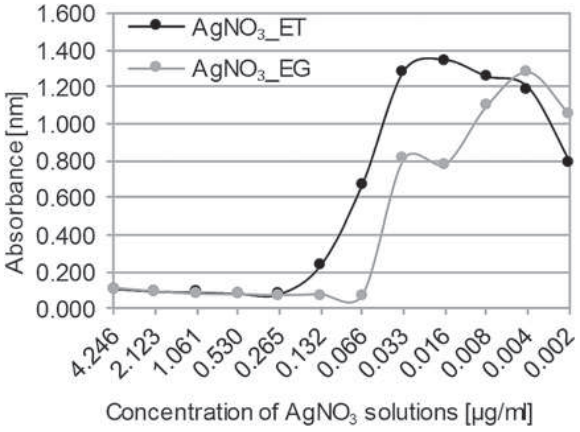

Figure 4: Graphical representation of antimicrobial effectiveness of $AgNO_3$ solution in absolute ethanol (ET) and ethylene-glycol (EG) on bacteria E. coli

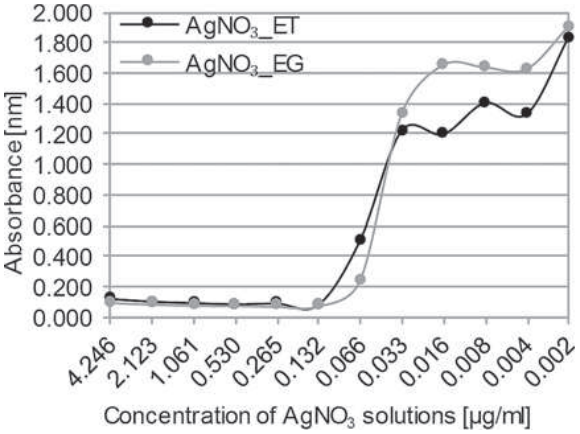

Figure 5: Graphical representation of antimicrobial effectiveness of $AgNO_3$ solution in absolute ethanol (ET) and ethylene-glycol (EG) on bacteria S. aureus

For bacteria *E. coli* minimal inhibitory concentration of $AgNO_3$ solution in absolute ethanol is 0.265 µg/ml and of $AgNO_3$ solution in ethylene-glycol 0.066 µg/ml. According to the obtained results, higher antimicrobial efficacy on *E. coli* is exhibited by the solution of silver nitrate in ethylene-glycol, rather than in absolute ethanol. The MIC value of $AgNO_3$ solution in ethanol and in ethylene-glycol for *S. aureus* is 0.132 µg/ml. From MIC values, it is visible that a higher concentration of $AgNO_3$ solution in ethylene-glycol is needed for inhibition of *S. aureus* than for *E. coli*. Based on the obtained data it can be concluded that silver nitrate is an excellent antimicrobial agent that exhibits excellent antimicrobial effectiveness even at very low concentrations. Although both solutions demonstrate great results, a more effective solution for both bacterial species proved to be $AgNO_3$ solution in ethylene-glycol.

3.3 Antimicrobial efficacy of treated samples

The testing results of antibacterial effectiveness of the treated samples for bacterial species *E. coli* and *S. aureus* are presented in Figures 6–9.

From the results, a positive effect of all treated samples for tested bacterial species is visible and it grows with the incubation time. The untreated textile samples as well as those treated only with HMDSO and AAC show slight antibacterial effect in time. It suggests that the mentioned samples are not a sufficient nutrient for large-scale growth and propagation of tested bacterial strains in defined time.

The best antibacterial effect for lyocell samples against *E. coli* was achieved after the treatment with $AgNO_3$ solution in ethylene-glycol using HMDSO as a precursor. A reduction of 75.2% of *E. coli* colonies after 18 hours of incubation was achieved. A 61.8% reduction of *E. coli* was obtained after 18 hours of incubation with the treatment of lyocell with $AgNO_3$ solution in ethylene-glycol and pretreatment with AAC.

For modal samples, the highest inhibition was attained after 18 hours of incubation after the treatment with $AgNO_3$ solution in absolute ethanol both using AAC (68.2%) and HMDSO (69.1%) as precursors.

In previous research [13], it was found that the application of HMDSO as a precursor enhanced the adsorption of silver particles onto cellulose providing higher antibacterial effect. According to the

obtained results for *E. coli*, both AAC and HMDSO proved to be suitable for the application as precursors using plasma process, with HMDSO as a slightly more efficient agent.

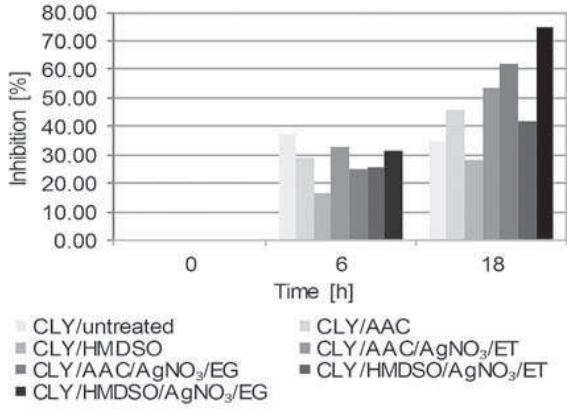

Figure 6: Inhibition of E. coli of treated CLY samples

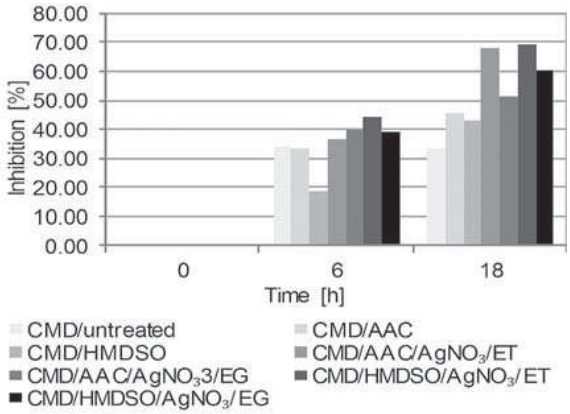

Figure 7: Inhibition of E. coli of treated CMD samples

The results of antibacterial effect against *S. aureus* (Figures 8 and 9) are not so uniform for the tested samples and inhibition is lower. The results for two treated samples that exhibit maximum inhibition (CLY/AAC, 6 hours of incubation and CMD/AAC/$AgNO_3$/EG, 18 hours of incubation) have a high degree of deviation and are inconsistent so they should be taken into account with caution and double checked. Besides them, the highest reduction of *S. aureus* for both lyocell and modal samples was achieved after the treatment with $AgNO_3$ solution in ethylene-glycol and HMDSO as a precursor (18 hours of incubation). Feng et al. [10] conducted a study of antibacterial effect of silver ions on *E. coli* and *S. aureus* and observed higher resistance of *S. aureus* than *E. coli*, which suggests a stronger defense system against silver ions. Because of its greater resistance, the application of higher concentration of agent should be considered.

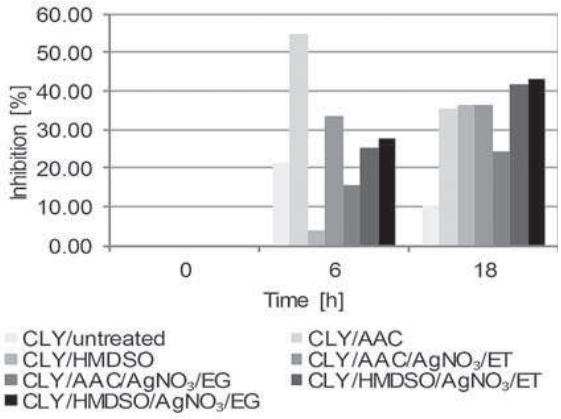

Figure 8: Inhibition of S. aureus of treated CLY samples

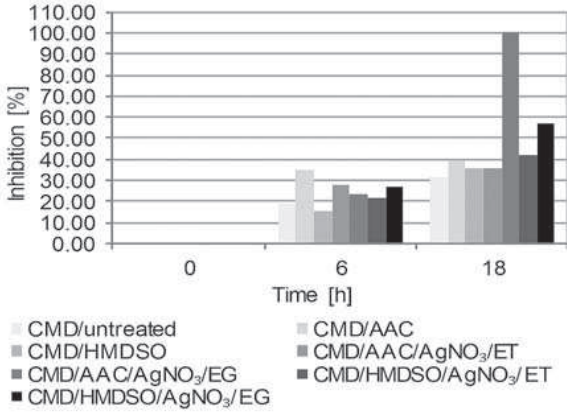

Figure 9: Inhibition of S. aureus of treated CMD samples

4 Conclusion

According to the obtained results, the following conclusions can be made:

- Plasma treatment certainly contributes to successful adsorption of chemical agents on man-made cellulosic fibers.
- From SEM micrographs, a significant presence of precursors is evident on the entire fiber surface, which confirms the effectiveness of the applied PE-CVD process.
- SEM micrographs also show the presence of silver particles on a treated fiber surface, which suggests the applied PDP-Ar process was conducted successfully.
- Based on the data for minimal inhibitory concentration of $AgNO_3$ solutions, it can be concluded

that silver is an excellent antimicrobial agent that exhibits great antimicrobial effectiveness even at very low concentrations.

- According to MIC values, $AgNO_3$ solution in ethylene-glycol proved to be a slightly more effective solution for both tested bacterial species.
- Highest inhibition for lyocell samples against *E. coli* was achieved after the treatment with $AgNO_3$ solution in ethylene-glycol using HMDSO as a precursor after 18 hours of incubation (75.2%).
- Highest inhibition for modal samples against *E. coli* was achieved after the treatment with $AgNO_3$ solution in absolute ethanol using HMDSO as a precursor after 18 hours of incubation (69.1%).
- Results of antibacterial effect against *S. aureus* obtained by time kill assay are not so uniform for the tested samples and the inhibition is lower. The reason may be a higher resistance of such bacteria to silver ions.
- Overall, both AAC and HMDSO proved to be suitable for the application as precursors using PE-CVD process, with HMDSO as a slightly more efficient agent.

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