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Vpliv velikosti delcev srebra na baktericidno učinkovitost celuloznih vlaken

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Influence of Particle Size of The Silver on Bactericidal Activity of the Cellulose Fibres

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Abstract

The aim of the study was to determine the influence of the particle size of silver in different antimicrobial agents on its bactericidal activity. Three commercial products were used, a dispersion of silver chloride (agent Ag-1), an elemental nanosilver in the form of a powder (agent Ag-2) and a colloidal silver (agent Ag-3). A dispersion of the agent Ag-2 was prepared just before its use. The agents were applied on cotton fabric according to the exhaustion method. As determined by scanning electron microscopy, the size of the silver particles in dispersion as well as on the finished samples of the fabric was classified as follows: agent Ag-1 ≈ agent Ag-3 >> agent Ag-2. The concentration of silver on the fibres was determined by the inductively coupled plasma mass spectroscopy and amounted to 138 mg/kg for agent Ag-1, 116 and 350 mg/kg for agent Ag-2 and 130 mg/kg for agent Ag-3. The bactericidal activity of the finishes was studied by bacterial reduction for the bacteria species Escherichia coli. The results showed that at resembling concentrations on the fibres, agents Ag-1 and Ag-3 caused a complete reduction of growth of Escherichia coli,

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Izvleček

Namen raziskave je bil določiti vpliv velikosti delcev srebra in srebrovih spojin v različnih tržnih produktih na njihovo baktericidno učinkovitost. Uporabljeni so bili trije tržni produkti, in sicer disperzija srebrovega klorida (sredstvo Ag-1), elementno nanosrebro v prahu (sredstvo Ag-2) in koloidno srebro (sredstvo Ag-3). Disperzija sredstva Ag-2 je bila pripravljena tik pred uporabo. Sredstva so bila nanesena na bombažno tkanino po izčrpalnem postopku. Delci srebra tako v disperziji kot tudi na apretiranih vzorcih tkanine, ki so bili preučevani z vrstično elektronsko mikroskopijo, so bili po velikosti razvrščeni na naslednji način: sredstvo Ag-1 ≈ sredstvo Ag-2-a >> sredstvo Ag-3. Koncentracija srebra na vlaknih je bila določena z masno spektrometrijo z vzbujanjem v induktivno sklopljeni plazmi in je za sredstvo Ag-1 znašala 138 mg/kg, za sredstvo Ag-2 116 in 350 mg/kg ter za sredstvo Ag-3 130 mg/kg. Baktericidne lastnosti apretur so bile določene na podlagi bakterijske redukcije za bakterijsko vrsto *E. coli*. Iz rezultatov raziskave je bilo razvidno, da sta sredstvi Ag-1 in Ag-3 pri podobnih koncentracijah povzročili popolno redukcijo rasti *E. coli*, sredstvo Ag-2 pa le 36 % redukcijo. Slednja se tudi po trikratnem povečanju koncentracije srebra ni povečala do te mere, da bi presegla 60 %. Iz rezultatov sledi, da na protimikrobno aktivnost srebra v preučevanih sredstvih ne vplivata le velikost delcev in njihova koncentracija, temveč tudi kemijska oblika srebra.

Ključne besede: bombažna tkanina, protimikrobna apretura, srebro, oblika srebra, bakterijska redukcija.

while only 36% of bacterial reduction was determined for agent Ag-2. Even after increasing the concentration of silver by three times, the bacterial reduction did not increase to such an extent to exceed 60%. Therefore, it can be concluded that the antibacterial activity of silver in the studied agents it is not influenced only by the particle size and their concentration, but also by the chemical form of silver.

Keywords: cotton fabric, antimicrobial finishing, silver, silver form, bacterial reduction.

1 Introduction

Heavy metals take an important place among antimicrobial agents. Namely, due to their inorganic nature, the probability of microorganisms to develop a resistance to such substances is unlikely [1]. Among heavy metals, silver is the most represented and studied, even though copper, zinc and cobalt are antimicrobially active as well [2–13]. Silver acts biocidally on many different microorganisms, such as Gram-positive and Gram-negative bacteria, fungi, protozoa and certain viruses. Due to a broad spectrum of antimicrobial activity, biologic compatibility and low toxicity, silver is being used in many economic fields and is also highly valued in the production of textiles with antimicrobial properties [14–38].

Silver can be found in many different forms on the market, mostly as hardly soluble salts, such as AgCl and $AgNO_3$, as elemental nanosilver with different particle dimensions or as colloidal silver. While commercially available products of silver salts and colloidal silver are stable dispersions or colloidal solutions, elemental silver, which exists in the form of a powder, needs to be preliminary dispersed in water. Products differ among each other in particle size and in the concentration of silver which directly influences their antimicrobial activity.

It can be seen from literature that silver ions, Ag^+, as well as silver nanoparticles Ag NPs, act antimicrobially [3, 4, 10, 13, 21, 22, 26, 30, 39–41]. Namely, the Ag^+ ions are being released by the dissociation of the silver salt in water, such as (Equation 1), or by the oxidation of particles of elemental silver in the presence of water and

1 Uvod

Med protimikrobnimi sredstvi zavzemajo težke kovine pomembno mesto, saj je zaradi njihove anorganske narave verjetnost prilagoditve mikroorganizmov nanje majhna [1]. Čeprav so protimikrobno aktivni baker, cink in kobalt, je med težkimi kovinami daleč najširše zastopano in preučevano srebro [2–13]. Srebro deluje biocidno na mnoge mikroorganizme, kot so grampozitivne in gramnegativne bakterije, glive, protozoji ter nekateri virusi. Zaradi širokega spektra delovanja, biološke kompatibilnosti in majhne toksičnosti za ljudi [14] sega uporaba srebra na različna gospodarska področja, pri čemer se je zelo uveljavilo tudi pri izdelavi tekstilij s protimikrobnimi lastnostmi [14–38].

Na tržišču je srebro kot protimikrobno sredstvo v različnih oblikah, največkrat kot težko topna sol, na primer AgCl in $AgNO_3$, kot elementno nanosrebro različnih dimenzij ali kot koloidno srebro. Medtem ko so tržni produkti srebrovih soli in koloidnega srebra stabilne disperzije oziroma koloidne raztopine, je potrebno elementno srebro, ki je v prahu, predhodno dispergirati v vodi. Produkti se med seboj razlikujejo v velikosti delcev in koncentraciji srebra, kar neposredno vpliva na njihovo protimikrobno učinkovitost.

Iz literaturnih virov je razvidno, da protimikrobno delujejo tako srebrovi kationi, Ag^+, kot nanodelci srebra, Ag NPs [3, 4, 10, 13, 21, 22, 26, 30, 39–41]. Ag^+ se sproščajo pri disociaciji srebrove soli, raztopljene v vodi, na primer:

$$AgCl_{(aq)} \rightarrow Ag^+_{(aq)} + Cl^-_{(aq)} \quad (1)$$

ali pri oksidaciji delcev elementnega srebra v prisotnosti vode in kisika, kot prikazuje naslednja reakcija [40, 41]:

$$O_{2(aq)} + 4H_3O^+ + Ag_{(s)} \rightarrow 4Ag^+_{(aq)} + 6H_2O \quad (2)$$

Ag NPs so v vodi netopni delci s premerom, manjšim od 100 nm. Pridobivajo se z raztapljanjem srebrovih soli (največkrat $AgNO_3$) in naknadno redukcijo srebrovih ionov z ustreznim reducentom, pri čemer poteče reakcija [3, 4, 21, 22, 26, 30]:

$$Ag^+_{(aq)} + 1e^- \rightarrow Ag^o_{(s)} \quad (3)$$

Na tak način nastane koloidno srebro, ki je v vodi v obliki stabilne disperzije oziroma koloidne raztopine.

Mehanizmi protimikrobne aktivnosti Ag^+ in Ag NPs, ki so predstavljeni v literaturnih virih, se med seboj le malo razlikujejo. Viri namreč razlagajo, da lahko Ag^+ in Ag NPs tvorijo privlačne interakcije z negativno nabito celično membrano bakterij, zaradi majhnosti delcev oziroma kationov pa prodrejo tudi v notranjost celice, kjer se vežejo na tiolne skupine encimov in nukleinske kisline [3, 6, 10, 13, 39, 42]. Vezanje srebra na mikroorganizem vpliva tako na strukturo in prepustnost membrane, ki obdaja celico mikroba, kot tudi na normalne metabolične in reprodukcijske aktivnosti celice mikroba, kar povzroči njeno uničenje. Iz rezultatov dosedanjih raziskav lahko razberemo, da so Ag NPs na splošno protimikrobno učinkovitejši kot Ag^+ [43]. Njihova aktivnost je neposredno odvisna od morfologije delcev. Manjši Ag NPs imajo večjo specifično površino, s katero lahko tvorijo interakcije, in so zato protimikrobno učinkovitejši kot večji Ag NPs [41, 42, 44, 45]. Če so delci dovolj majhni, ne tvorijo le interakcij s površino celične membrane mikroorganizma, temveč celo prehajajo v notranjost celice [42]. V notranjosti celice se lahko v prisotnosti oksidantov oksidirajo v Ag^+, kar dodatno prispeva k njihovemu baktericidnemu delovanju [42, 46].

Raznolikost izbire tržnih produktov protimikrobnih sredstev na podlagi srebrovih spojin in elementnega srebra ter predvsem pomanjkanje literaturnih podatkov o primerjavi njihovih protimikrobnih učinkovitosti sta nas spodbudila, da smo v raziskavi preučili vpliv velikosti delcev in koncentracije srebra na tekstilnih vlaknih na njegovo baktericidno učinkovitost. V raziskavo smo vključili tri tržne produkte v primerljivih koncentracijah, in sicer disperzijo AgCl, nanosrebro v prahu velikosti delcev 30 nm in koloidno srebro.

oxygen, as it is shown in the next reaction [40, 41] (Equation 2).

In water Ag NPs are insoluble particles with a diameter smaller than 100 nm. They are acquired by dissolving the silver salts (most often $AgNO_3$) and the subsequent reduction of silver ions by the proper reducing agent following this reaction [3, 4, 21, 22, 26, 30] (Equation 3).

In this way the colloidal silver, which is found in water in the form of a stable dispersion or a colloidal solution, is formed.

Only a small difference can be perceived in the mechanisms of the antimicrobial activity of the Ag^+ and Ag NPs in literary sources. Namely, both Ag^+ as well as Ag NPs can form attractive interactions with negatively charged membranes of bacteria. Moreover, due to the small size of the particles or cations, they can also penetrate inside the cell where they bond with thiol groups of enzymes or nucleic acid [3, 6, 10, 13, 39, 42]. The bonding of silver on microorganism influences the structure and the permeability of the cell membrane, as well as its normal metabolic and reproduction activity, which eventually cause microorganism's death. From the results of previous studies, it can be seen that Ag NPs are generally more antimicrobially active compared to Ag^+ [43]. The activity of Ag NPs is directly influenced by the morphology of the particles. Hence, smaller particles have larger specific surface area by which they can form interactions and are therefore more antimicrobially active compared to greater Ag NPs with a smaller specific surface area [41, 42, 44, 45]. If the particles are small enough, not only, can they form interactions with the surface of the cell membrane, but they can also penetrate inside the cell, where they oxidise to Ag^+ in the presence of oxidant agents, which additionally contributes to their bactericidal activity [42, 46].

The diverse choice of commercial antimicrobial products based on silver compounds and especially the lack of data comparing their antimicrobial activities in the literature encouraged us to study the influence of particle size and concentration of silver on textile fibres on its bactericidal activity. In the research, three commercial products in comparable concentrations were included, these are a dispersion of AgCl,

2 Eksperimentalni del

2.1 Tkanina in apreturna sredstva

V raziskavi smo uporabili 100 % bombažno tkanino v vezavi platno s ploščinsko maso 164 g/m^2, gostoto osnove 28 niti/cm in gostoto votka 24 niti/cm. Tkanina je bila predhodno beljena s H_2O_2, mercerizirana v raztopini NaOH in nevtralizirana z razredčeno CH_3COOH.

Med protimikrobnimi sredstvi smo izbrali tržni produkt iSys Ag (BEZEMA, Švica), ki je disperzija srebrovega klorida in se nanaša v kombinaciji z organsko-anorganskim zamreževalom iSys MTX istega proizvajalca (sredstvo Ag-1), Silver Nano Powder NP-30 (Ames Goldsmith Corporation), ki je elementno srebro v prahu s povprečno velikostjo delcev 30 nm (sredstvo Ag-2), in Ionosil (Ion Silver, Švedska), ki je koloidno srebro s povprečno velikostjo delcev manj kot 10 nm (sredstvo Ag-3). Sredstvo Ag-1 smo pripravili v koncentraciji 3 g/l iSys Ag in 15 g/l iSys MTX na tak način, da smo tržna produkta ustrezno razredčili z vodo. Disperzijo sredstva Ag-2 smo pripravili v dveh koncentracijah, in sicer 20 (sredstvo Ag-2-a) in 100 mg/l (sredstvo Ag-2-b) produkta Silver Nano Powder NP-30 ob dodatku 2 g/l dispergirnega sredstva Setamol WS (BASF, Nemčija), ki je kondenzacijski produkt naftalen sulfonata s formaldehidom. Da bi bila disperzija čim stabilnejša, smo jo pred uporabo 10 minut obdelovali v ultrazvočni kadički pri frekvenci 50 kHz in temperaturi 25 °C. Sredstvo Ag-3 smo uporabili koncentrirano in ga predhodno nismo redčili z vodo.

nanosilver in the form of powder with particle size of 30 nm and colloidal silver.

2 Experimental

2.1 Fabric and finishing agents

Plain-weave 100% cotton fabric with a mass of 164 g/m², warp density of 28 yarns/cm and weft density of 24 yarns/cm was used in the experiments. In a pre-treatment process the fabric was bleached and mercerised.

Among antimicrobial agents, commercial products iSys Ag (BEZEMA, Switzerland), which is a dispersion of silver chloride and is applied in a combination with inorganic-organic binder iSys MTX (BEZEMA, Switzerland) (agent Ag-1), Silver nano Powder NP-30 (Ames Goldsmith Corporation), which is an elemental silver in the form of powder with average particle size of 30 nm (agent Ag-2) and Ionosil (Ion Silver, Sweden), which is a colloidal silver with average particle size smaller than 10 nm (Agent Ag-3), were chosen. Agent Ag-1 was prepared by making a proper water dilution of a mixture composed of 3g/l of iSys Ag and 15 g/l of iSys MTX. A dispersion of agent Ag-2 was prepared by using two concentrations, i. e. 20 (agent Ag-2-a) and 100 mg/l (agent Ag-2-b) of Silver Nano Powder NP-30 with the addition of 2 g/l of dispersing agent Setamol WS (BASF, Germany), which is a condensation product of naphthalen sulphonate and formaldehyde. In order to obtain a dispersion with silver particles as small as possible, the latter was treated with an ultra sound for 10 minutes at a frequence of 50 Hz and a temperature of 25 °C. An agent Ag-3 was used as supplied.

2.2 Application of finishing agents on Si-waffer and cotton fabric

Silicon (Si) wafers were coated with dispersions of agents Ag-1, Ag-2 and Ag-3 according to the deep-coating technique. Afterwards, the Si-wafers were air dried and heat treated for 5 minutes at 150 °C.

The finishing of cotton fabric with the agents Ag-1, Ag-2 and Ag-3 of proper concentration were carried out by the exhaustion method in a Launder-ometer for 30 minutes in a liquid ratio of 1:50 at a temperature of 25 °C. Afterwards,

2.2 Nanos apreturnih sredstev na silicijevo ploščico in bombažno tkanino

Silicijeve (Si) ploščice smo potopili v disperzije sredstev Ag-1, Ag-2-a in Ag-3, jih počasi izvlekli, posušili in segrevali 5 minut pri 150 °C v sušilniku.

Apretiranje bombažne tkanine s sredstvi Ag-1, Ag-2 in Ag-3 ustreznih koncentracij smo izvedli po izčrpalnem postopku v Launder-ometru 30 minut v kopelnem razmerju 1 : 50 pri temperaturi 25 °C. Po impregniranju smo vzorce oželi na dvovaljčnem fularju s 100 % ožemalnim učinkom in jih posušili v razpenjalnem sušilniku pri temperaturi 120 °C. Vzorec, apretiran z disperzijo srebrovega klorida, smo naknadno še kondenzirali 1 minuto pri temperaturi 150 °C. Pri tej temperaturi je prišlo do zamreženja organsko-anorganskega zamreževala.

2.3 Metode preiskav

Vrstična elektronska mikroskopija (SEM)

Morfološke lastnosti apreturnih filmov sredstev Ag-1, Ag-2 in Ag-3 na Si-ploščici in bombažni tkanini smo določili z uporabo vrstičnega elektronskega mikroskopa JEOL JSM 5800. Da bi preprečili nabijanje električno neprevodnih delov vzorca, smo na površino vzorca nanesli tanko plast ogljika (približno 20 nm). Analizo smo izvedli z uporabo energije elektronov 10 keV, gostoto toka elektronov 200 do 500 pA in nagibom vzorca 35°. Topografijo površine vzorca in plast na prelomu vzorca smo opazovali tako s sekundarnimi (SE) kot tudi povratno sipanimi primarnimi elektroni (BSE). Sliko, ki je nastala z BSE-elektroni, smo uporabili za razlikovanje nanesenih apreturnih delcev od bombažnih vlaken in drugih nečistoč.

Masna spektroskopija z induktivno sklopljeno plazmo (ICP-MS)

Koncentracijo srebra na apretiranih vzorcih tkanine smo določili z ICP-MS na spektrofotometru Perkin Elmer SCIEX Elan DRC. Vzorec velikosti 0,5 g smo pripravili v mikrovalovnem sistemu Milestone s kislinsko dekompozicijo s 60 % HNO_3 in 30 % H_2O_2.

Bakterijska redukcija

Bakterijsko redukcijo vzorcev tkanine, apretiranih s sredstvi Ag-1, Ag-2 in Ag-3, smo izvedli za bakterijsko vrsto *Escherichia coli* (ATCC 25922) po standardni metodi AATCC 100-1999. Vzorec tkanine smo prenesli v erlenmajerico, ga prelili s suspenzijo bakterij določene koncentracije in inkubirali pri temperaturi 37 °C 24 ur. Po inkubaciji smo vzorec prelili s 100 ml sterilne destilirane vode, 1 minuto intenzivno stresali in suspenzijo ustrezno razredčili. Razredčino smo razmazali na agar plošče in inkubirali 24 ur pri 37 °C. Po inkubaciji smo prešteli bakterijske kolonije in izračunali bakterijsko redukcijo, *R*, iz naslednje enačbe:

$$R = \frac{B - A}{B} \times 100\ [\%] \qquad (4)$$

the samples were wrung by a wet-pick-up of 80 ± 1% and dried at 120 °C. The sample finished by a dispersion of silver chloride was subsequently cured at 150 °C for 1 minute in order to achieve the crosslinking of the inorganic-organic binder.

2.3 Analysis and measurements

Scanning electron microscopy (SEM)

The morphology of the finishing films of agents Ag-1, Ag-2 and Ag-3 on Si wafer and cotton fabric was determined by the JEOL JSM 5800 scanning electron microscope. The samples were coated with ≈ 20-nm-thick carbon layer to ensure sufficient electrical conductivity and to avoid charging effects. Analyses were performed using a 10-keV electron beam, 200 to 500 pA beam current and X-ray spectra acquisition under a 35° take-off angle. SEM micrographs were recorded using both secondary electron (SE) and backscattered electron (BSE) imaging modes. BSE compositional (Z-contrast) imaging was applied to emphasize and expose the difference between the added particles and the cotton fibre-matrix.

Inductively coupled plasma mass spectroscopy (ICP-MS)

The concentration of silver on finished cotton samples was determined by ICP-MS on a Perkin Elmer SCIEX Elan DRC spectrophotometer. A sample of 0.5 g was prepared in the Milestone microwave system with an acid decomposition using 60% HNO_3 and 30% H_2O_2.

A je število bakterijskih kolonij v suspenziji po 24 urah stika suspenzije z vzorcem apretirane tkanine in *B* število bakterij v suspenziji po 24 urah stika suspenzije z neapretirano tkanino. Za zadovoljivo protimikrobno delovanje sredstva mora vrednost *R* preseči 60 %. Za vsak vzorec tkanine smo opravili dve ponovitvi.

3 Rezultati z razpravo

Na sliki 1 sta prikazana SEM-posnetka apreturnih filmov sredstev iSys Ag in Ag-2-a na Si-ploščicah. Na njih je jasno vidna prisotnost kristalov AgCl v sredstvu Ag-1 (slika 1A) kot tudi manjših in večjih aglomeratov nanodelcev srebra v primeru sredstva Ag-2 (slika 2B). Ker pri sredstvu Ag-3 na Si-ploščici nismo mogli opaziti nobenih sledi srebra, smo iz tega sklepali, da je bil nanos delcev koloidnega srebra na Si-ploščico premajhen, da bi delce lahko opazili pod mikroskopom.

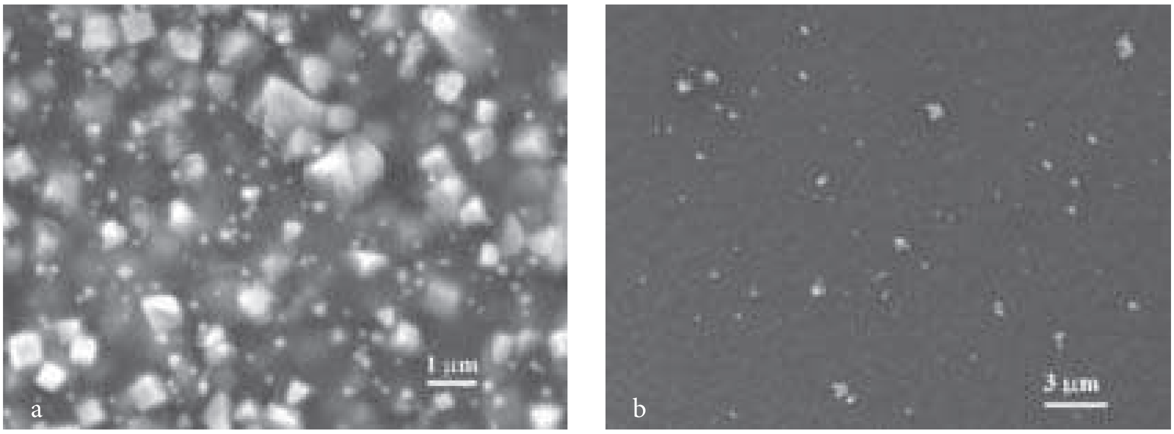

Figure 1: SEM images of Ag-1 (A) and Ag-2-a (B) films on the Si-wafers.

SEM-posnetki so razkrili prisotnost srebra tudi na celuloznih vlaknih (slika 2). V primeru sredstva Ag-1 so bili delci kristalov AgCl krogelne oblike in velikosti od 100 do 500 nm ter tudi večji (slika 2A). Za sredstvo Ag-2-a smo tako kot na Si-ploščici tudi na vlaknih poleg manjših delcev velikosti od 100 do 300 nm zasledili večje skupke, ki so v nekaterih primerih dosegli celo velikosti od 1 do 5 µm (slika 2B). Slednji so nastali kljub uporabi dispergirnega sredstva in obdelovanju impregnirne kopeli z ultrazvokom. Na slikah 2A in 2B lahko vidimo, da je porazdelitev srebrovih delcev po vlaknih dokaj neenakomerna, še posebej v primeru disperzije nanosrebra v prahu, in to kljub temu, da smo disperziji nanesli po izčrpalnem postopku, ki v primerjavi z impregnirnim postopkom omogoča boljše kroženje impregnirne kopeli okrog bombažne tkanine in s tem dosego enakomernejšega nanosa. Sredstvo Ag-3 se je po vlaknih razporedilo veliko enakomerneje (slika 2C) kot sredstvi Ag-1 in Ag-2. Tudi velikost delcev je bila veliko manjša in je le v redkih primerih presegla 20 nm. Na podlagi SEM-posnetkov

Reduction of bacteria

The antibacterial activity of the samples treated by the finishing agents Ag-1, Ag-2 and Ag-3 was determined according to the AATCC 100-1999 standard method, for bacterium Escherichia coli (ATCC 25922). A sample of the finished cotton was put into the Erlenmeyer flask and inoculated with a nutrient broth culture containing a certain amount of bacteria and incubated at 37 °C for 24 hours. After incubation, the bacteria were eluted from the swatches by shaking them in 100 ml of neutralizing solution for 1 minute. After making serial dilutions, the suspensions were plated on nutrient agar and incubated at 37 °C for 24 hours. Afterwards, the number of bacteria forming units (CFU) was counted, and the reduction of bacteria, R, was calculated from (Equation 4), where A is the CFU recovered from the inoculated finished cotton sample swatch in the jar incubated over the desired contact period (24 hours), and B is the CFU recovered from the inoculated unfinished cotton sample swatch in the jar incubated over the desired contact period (24 hours). In order to achieve satisfying antimicrobial activity the value R must exceed 60%. For each finished cotton fabric, two treatments were performed.

3 Results and discussion

In figure 1, SEM images of coating films of agents iSys Ag and Ag-2 on Si-wafers are shown. In the case of agent Ag-1, the presence of AgCl crystals is clearly seen (figure 1A), while in the case of agent Ag-2 smaller and larger agglomerates of silver nanoparticles were observed (figure 1B). In the case of agent Ag-3 no trace of silver was noticed on the Si-wafer. Therefore, it was inferred that the application of particles of colloidal silver on Si-wafer was too small to be seen by a microscope. Namely, according to the manufacturer's declaration, the concentration of silver in the colloidal solution was only 10 mg/kg.

SEM images revealed the presence of silver on cellulose fibres as well (figure 2). In the case of agent Ag-1 the particles of AgCl crystals were spherical in shape with the size ranging from 100 to 500 nm (figure 2A). Larger agglomerates

smo lahko brez večjih napak velikosti delcev srebra na apretiranih tkaninah razvrstili na naslednji način: sredstvo Ag-1 ≈ sredstvo Ag-2-a >> sredstvo Ag-3.

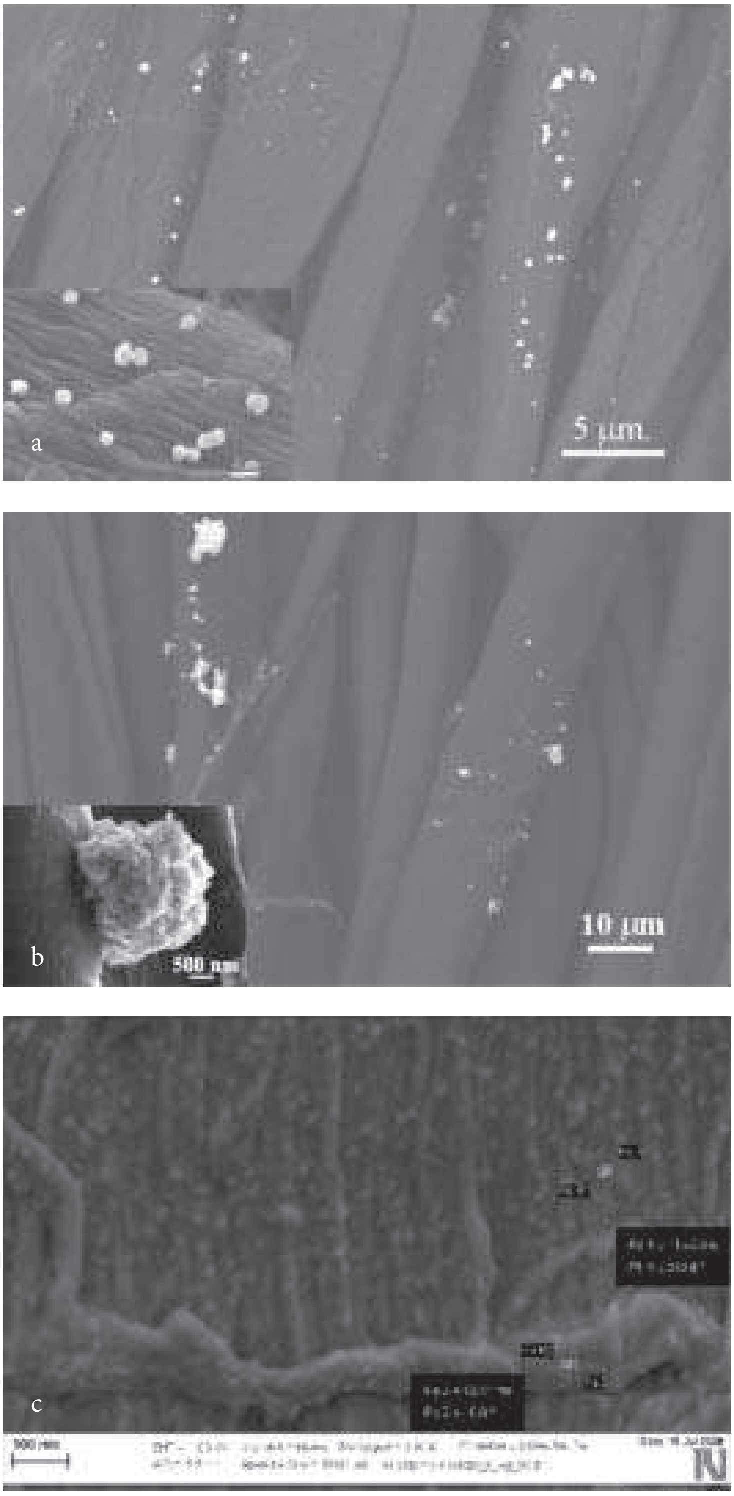

Figure 2: SEM images of a cotton fabric finished by agents Ag-1 (A), Ag-2-a (B) and Ag-3 (C).

ranging from 1 to 5 μm were observed for agent Ag-2 on Si-wafer as well as on cotton fibres beside smaller particles ranging from 100 to 300 nm (figure 2B). They were formed despite the use of dispersing agent and treatment of finishing bath by ultrasound. From figures 2A and 2B it can be also seen that the distribution of silver particles on textile fibres was rather non-uniform, which especially holds for dispersion of nanosilver in the form of a powder and it occurred even though an exhausting method of application was used in both cases. Namely, compared to the impregnation, the exhausting method enables better circulation of finishing bath around the cotton fabric which reflects in achieving a more uniform application. On the other hand, compared to the agents Ag-1 and Ag-2, the application of the agent Ag-3 was more uniform. In this case the size of the particles was also much smaller and it rarely exceeded 20 nm. Therefore, based on SEM images, the particle size of the silver on the finished fabrics can be classified as follows: agent Ag-1 ≈ agent Ag-2 >> agent Ag-3.

The concentration of silver on the finished cotton samples, which was determined by ICP-MS analysis, reached 138 mg/kg for agent Ag-1, 116 mg/kg for agent Ag-2-a and 130 mg/kg for agent Ag-3. In spite of the similar concentration of silver on the fibres, the results of antimicrobial activity of the studied agents varied among each other surprisingly. While agents Ag-1 and Ag-3 caused perfect 99–100% growth reduction of bacteria E. coli, the bacterial reduction of the sample finished by agent Ag-2 was only 36% (figures 3 and 4). Even after increasing the concentration of agent Ag-2-a on the fibres by three times (this was obtained by the use of agent Ag-2-b where the concentration of silver on the fibres was 350 mg/kg), the bacterial reduction did not change essentially and did not exceed the limit value for achieving satisfactory antimicrobial activity, i.e. 60% (figure 3). These results show that agent Ag-1 was far more antimicrobially active compared to agent Ag-2, even though comparable particle size of the silver was determined on the fibres by SEM analysis. Hence, it can be seen that in this case neither the size nor the concentration of the particles of silver have influenced their antimicrobial activity.

Z analizo ICP-MS apretiranih vzorcev tkanine smo določili koncentracijo srebra na celuloznih vlaknih, ki je znašala 138 mg/kg za sredstvo Ag-1, 116 mg/kg za sredstvo Ag-2-a in 130 mg/kg za sredstvo Ag-3. Kljub podobnim koncentracijam srebra na vlaknih so se rezultati protimikrobne učinkovitosti sredstev med seboj presenetljivo razlikovali. Medtem ko smo z uporabo sredstev Ag-1 in Ag-3 dobili odlično 99–100 % redukcijo rasti bakterije *E. coli*, pa je bila bakterijska redukcija vzorca, apretiranega s sredstvom Ag-2-a, le 36 % (sliki 3 in 4). Tudi po trikratnem zvišanju koncentracije tega sredstva na vlaknih, ki smo ga dosegli z uporabo sredstva Ag-2-b, ko je koncentracija znašala 350 mg/kg, se bakterijska redukcija ni bistveno povečala, saj je dosegla le vrednost 60 % (slika 3), ki predstavlja mejno vrednost za zadovoljivo protimikrobno zaščito. Iz teh rezultatov je razvidno, da je sredstvo Ag-1 protimikrobno veliko aktivnejše kot Ag-2, in to kljub temu, da smo za obe sredstvi s SEM-analizo določili primerljive velikosti delcev na vlaknih. Iz tega sledi, da v tem primeru niti velikost delcev niti koncentracija srebra nista tisti, ki bi odločilno vplivali na protimikrobno učinkovitost sredstev.

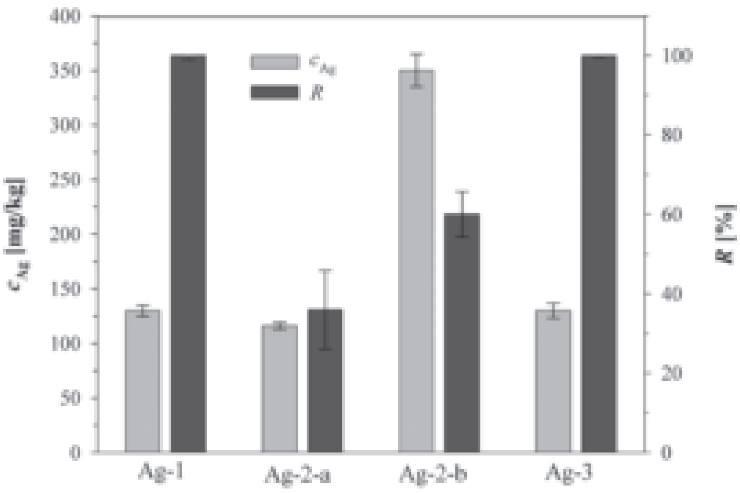

Figure 3: The concentration, c, of silver on the samples treated by the studied agents and the bacterial reduction, R, determined according to the AATCC 100-1999 Standard method for bacterium Escherichia coli.

Razlike v baktericidni aktivnosti sredstev Ag-1 in Ag-2 smo lahko smiselno razložili na podlagi razlik v njuni kemijski strukturi. Sredstvo Ag-1 je disperzija soli AgCl, sredstvo Ag-2 pa je disperzija elementnega srebra, Ag°. Medtem ko je za protimikrobno učinkovitost sredstva Ag-1 odločilna koncentracija Ag^+, ki se sprostijo s površine kristalov soli v prisotnosti vode (reakcija 1), je protimikrobna učinkovitost sredstva Ag-2 povezana tako z biocidnim delovanjem Ag NPs kot tudi s sproščanjem Ag^+ s površine elementnega srebra v reakciji oksidacije, ki poteče v prisotnosti vode in kisika (reakcija 2). Vendar pa so rezultati SEM-analize pokaza-

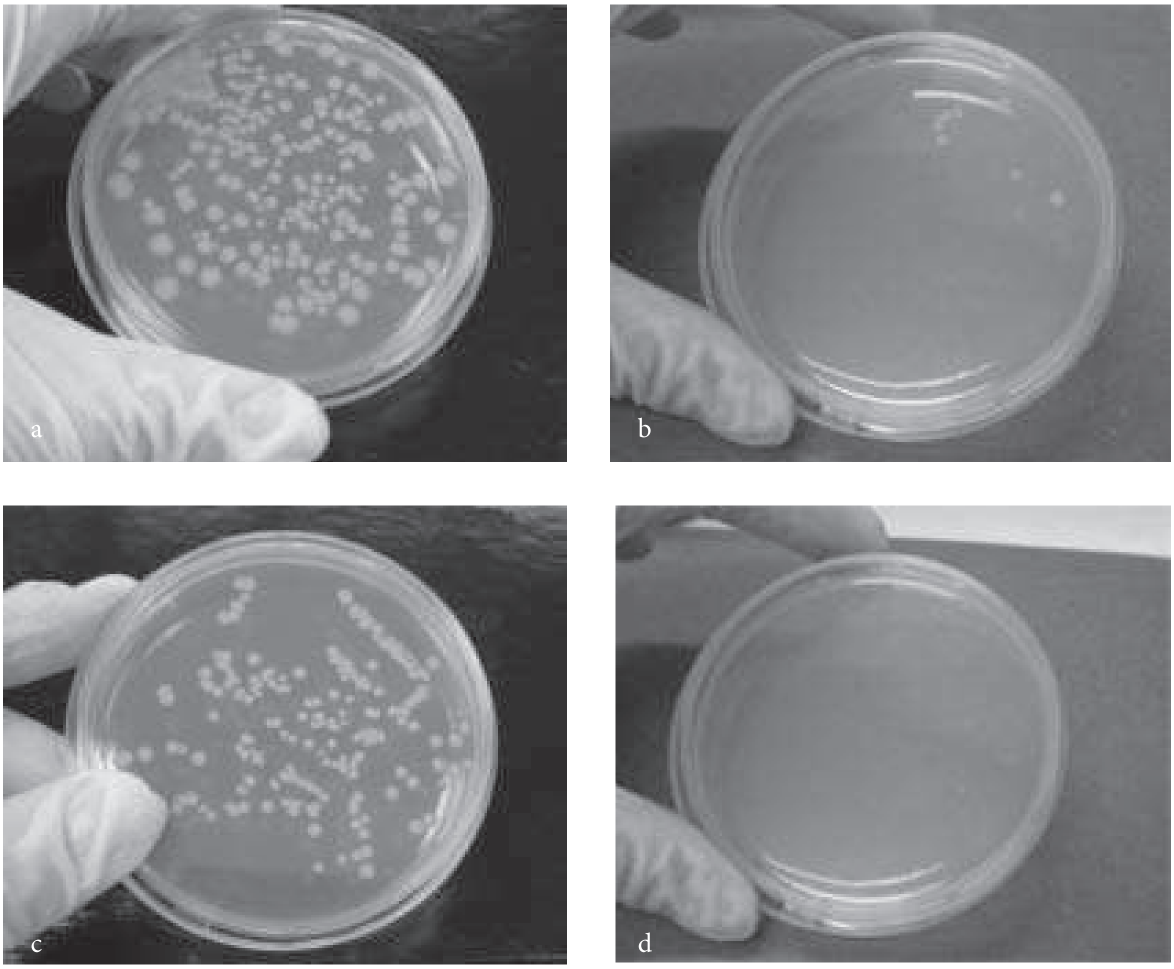

Figure 4: The number of colony forming units of bacterium Escherichia coli after 24-hours of incubation determined according to the AATCC 100-1999 Standard method. Samples: A – unfinished, B – agent Ag-1, C – agent Ag-2-a, D – agent Ag-3.

Differences in bactericidal activity of agents Ag-1 and Ag-2 were reasonably explained based on their dissimilar chemical form. Namely, agent Ag-1 was a dispersion of AgCl salt, while agent Ag-2 was a dispersion of elemental silver, Ag^0. Therefore, for the antimicrobial activity of agent Ag-1, the concentration of Ag^+ released from the surface of the crystal in the presence of water (reaction 1) is important, while the antimicrobial activity of agent Ag-2 is influenced by the biocidal activity of Ag NPs, as well as by releasing Ag^+ from the surface of elemental silver, which occurs in the reaction of oxidation in the presence of water and oxygen (reaction 2). However, the results of SEM analysis showed that when in dispersion or on the fibres, silver particles in agent Ag-2 strongly ag-

li, da so delci srebra v sredstvu Ag-2 tako v disperziji kot na vlaknih močno agregirali in da je bila velikost skupkov na vlaknih veliko večja od 30 nm, kot je bila deklarirana velikost delcev srebra v prahu. Tako veliki skupki Ag NPs prav gotovo ne morejo delovati protimikrobno, saj so rezultati dosedanjih raziskav [42, 44] pokazali, da se z naraščajočo velikostjo delcev protimikrobna aktivnost Ag NPs močno zmanjšuje in da lahko le Ag NPs s premerom, manjšim od 10 nm, tvorijo neposredne interakcije z bakterijami. V primeru sredstva Ag-2 je torej njegovo protimikrobno delovanje na vlaknih odvisno predvsem od sproščanja Ag^+ z njihove površine. Ker je bilo ugotovljeno, da je koncentracija sproščenih Ag^+, ki nastanejo v reakciji oksidacije, odvisna od specifične površine delcev [13, 39, 41], le-ta pa je obratno sorazmerna z velikostjo Ag NPs, je tvorba skupkov Ag NPs najverjetneje vzrok za slabo protimikrobno učinkovitost sredstva Ag-2. Zaradi majhne specifične površine skupkov Ag NPs je bila namreč koncentracija Ag^+ tako nizka, da ni dosegla kritične koncentracije inhibicije. To potrjuje-

glomerated, so their size was far larger than 30 nm as was the declared size of the silver particles in powder. Such large agglomerates surely cannot act antimicrobially. Namely, the results of previous studies [42, 44] have shown that by increasing the particle size, the antimicrobial activity of Ag NPs strongly decreases and that only Ag NPs with a diameter smaller than 10 nm can form interaction with the bacteria. Therefore, the antimicrobial activity of agent Ag-2 depends paramountly on releasing Ag^+ from its surface. Because it was found out that the concentration of released Ag^+ formed in the reaction of oxidation depends on the specific surface area of the particles [13, 39, 41], which is inversely proportioned with the size of Ag NPs, most likely the formation of agglomerates is the main reason for a weak antimicrobial activity of agent Ag-2. Due to a low specific surface area of the Ag NPs agglomerates, the concentration of Ag^+ was too low to reach the critical inhibitory concentration. This was confirmed by the results of the bacterial reduction, where even after increasing the concentration of elemental silver by three times, no significant increase of antimicrobial activity was obtained. At this point, it also has to be stressed out that the concentration of silver on the fibres was so high that it visually influenced the decrease of the whiteness index of the fabric [32]. In relation to this, even with a further increase of the concentration of Ag-2, no additional increase of its antimicrobial activity would be achieved. Namely, due to Van der Waals interaction among the Ag NPs their average aggregation number would increase, reflecting in a decrease of their specific surface area as well as in a decrease in the concentration of the released Ag^+. Therefore, when using elemental silver in form of powder, the preparation of stable dispersion in which aggregation of Ag NPs would not occur is more important than increasing the concentration of such agents. Concerning this, the choice of a proper dispersing agent is very important. In our research Setamol WS was used, since, according to the literature, the sodium salts of naphthalene sulfonate formaldehyde condensate enables the preparation of the most stable dispersion of silver nanoparticles in comparison to sodium dodecyl sul-

jo rezultati bakterijske redukcije, saj tudi po trikratnem povečanju koncentracije elementnega srebra na vlaknih nismo dosegli bistvenega povečanja protimikrobne aktivnosti. Pri tem je treba poudariti, da je bila koncentracija srebra na vlaknih tako visoka (350 mg/kg), da je vidno vplivala na zmanjšanje beline tkanine [32]. Glede na to tudi z nadaljnjim povečevanjem koncentracije sredstva Ag-2 ne bi dosegli povečanja protimikrobne učinkovitosti sredstva Ag-2, saj bi se s tem zaradi delovanja Van der Waalsovih sil med delci Ag NPs povečevalo njihovo povprečno agregacijsko število in posledično bi se zniževali specifična površina delcev ter koncentracija sproščenih Ag^+. Zato je pri uporabi elementnega srebra v prahu pomembnejša kot povečanje njegove koncentracije priprava stabilne disperzije, v kateri Ag NPs ne bi bili agregirani. Pri tem je izredno pomembna izbira ustreznega dispergirnega sredstva. V naši raziskavi smo se odločili za Setamol WS, saj smo iz literaturnih virov ugotovili, da v primerjavi z natrijevim dodecilsulfatom, sredstvom Tween 20 in cetiltrimetilamonijevim bromidom omogočajo dispergirna sredstva, ki so kondenzacijski produkt naftalen sulfonata s formaldehidom, pripravo najstabilnejše disperzije srebrovih nonodelcev [47]. Obstaja pa verjetnost, da Setamol WS z negativnim nabojem v raztopini kljub temu ni primerno dispergirno sredstvo, saj lahko zaradi prisotnosti Ag^+ na površini delcev elementnega srebra dodatno prispeva k tvorbi večjih skupkov. Glede na to ostaja priprava stabilnih disperzij Ag NPs še vedno pereč in nerešen problem.

Protimikrobna aktivnost sredstva Ag-3 je bila povsem primerljiva s protimikrobno aktivnostjo sredstva Ag-1 in veliko boljša kot pri sredstvu Ag-2 (sliki 3 in 4). Ti rezultati so potrdili že znano ugotovitev [13, 14, 40], da je biocidna aktivnost Ag NPs neposredno odvisna od njihove specifične površine, ki je obratno sorazmerna z velikostjo delcev. Ker je bila v primeru sredstva Ag-3 velikost Ag NPs veliko manjša (v povprečju 20 nm) kot pri sredstvu Ag-2, je bila njihova specifična površina veliko večja, kar je po pričakovanju močno povečalo biocidno aktivnost koloidnega srebra v primerjavi z elementnim nanosrebrom v prahu. Ker pa smo v primeru sredstva Ag-3 uporabili tržni produkt koloidnega srebra, ne moremo z gotovostjo trditi, da so v koloidni raztopini prisotni le Ag NPs, dobljeni z redukcijo srebrove soli (reakcija 3). Koloidno srebro se namreč lahko proizvaja tudi z elektrolizo, pri čemer so v koloidni raztopini prisotni tudi Ag^+. Ker je postopek izvedbe elektrolize veliko bolj preprost in cenejši od postopka redukcije srebrove soli, lahko iz tega sklepamo, da so v tržnem produktu uporabljenega koloidnega srebra prisotni tako Ag NPs kot Ag^+ v določenem koncentracijskem razmerju. To, ali so pri protimikrobnem delovanju aktivnejši in učinkovitejši prvi ali drugi, pa iz rezultatov raziskave ne moremo sklepati. Odlična protimikrobna aktivnost sredstva Ag-3 nakazuje, da Ag NPs in Ag^+ na vlaknih delujejo sinergistično.

phate, Tween 20 and cetyltrimethylammonium bromide [47]. Nevertheless, due to the negative charge in the solution, Setamol WS is probably not a suitable dispersing agent. Namely, in the presence of Ag^{+} on the surface of the particles of the elemental nanosilver Setamol WS can additionally contributes to the formation of larger agglomerates. Regarding this, the preparation of stable dispersions of Ag NPs still remains an urgent and unsolved problem.

The antimicrobial activity of agent Ag-3 was completely comparable with agent Ag-1 and much better than with agent Ag-2 (figures 3 and 4). These results confirmed the already known finding [13, 14, 40] that the biocidal activity of Ag NPs directly depends on their specific surface area which is inversely proportioned to the size of the particles. Because in the case of agent Ag-3 the size of Ag NPs was much smaller (≈ 20 nm) compared to agent Ag-2, their specific surface area was larger, which expectedly strongly increased the biocidal activity of the colloidal silver compared to the elemental silver in the form of powder. Since in the case of agent Ag-3 commercial product was used, it can not be guaranteed that only Ag NPs, formed by the reduction of silver salt, (reaction 3) were present in the colloidal solution. Hence, colloidal silver can be produced by electrolysis as well, where Ag^{+} ions are also present in the colloidal solution. Because the procedure of electrolysis is simpler and low-priced compared to the procedure of reduction of silver salt, it can be inferred that in the commercial product of colloidal silver which was used in our experiment, Ag NPs as well as Ag^{+} were present at a certain concentration ratio. From the results of our study it cannot be concluded whether the former or the latter are more antimicrobially effective, but a great bacterial reduction clearly shows that Ag NPs and Ag^{+} act on fibres synergistically.

4 Conclusions

In the study the bactericidal activity of three commercial products based on silver compounds applied on cellulose fibres in comparable concentration was studied. The size of silver chloride and elemental silver particles was

4 Zaključki

V raziskavi je bila preučevana baktericidna učinkovitost treh tržnih produktov, ki vsebujejo srebro v različnih kemijskih oblikah, na celuloznih vlaknih v primerljivih koncentracijah. Velikosti delcev srebrovega klorida in elementnega srebra na vlaknih so bile v povprečju od 100 do 500 nm in veliko večje od delcev koloidnega srebra, katerih velikost ni presegala 20 nm. Bakterijska redukcija je bila odlična 99 do 100 % v primeru srebrovega klorida in koloidnega srebra, za nanosrebro v prahu pa je znašala le 36 % in je tudi pri trikratnem povišanju koncentracije dosegla le vrednost 60 %, kar ni zadovoljivo. Iz teh rezultatov smo sklepali, da protimikrobna aktivnost preučevanih produktov ni bila odvisna le od koncentracije in velikosti delcev, temveč tudi od kemijske oblike srebra. V primeru srebrovega klorida so baktericidno delovali Ag^{+}, ki so se v prisotnosti vode sproščali s površine kristalov soli AgCl. Tudi v primeru nanosrebra v prahu je bilo protimikrobno delovanje omejeno le na sproščanje Ag^{+}, saj so nanodelci srebra na vlaknih agregirali v tako velike skupke, da protimikrobno delovanje Ag NPs ni bilo opaženo. Boljše baktericidno delovanje bi verjetno opazili le, če bi bila njihova velikost manjša od 10 mn. Za sproščanje Ag^{+} s površine elementnega srebra je v reakciji oksidacije potrebna velika specifična površina delcev, da bi lahko koncentracija sproščenih Ag^{+} dosegla kritično koncentracijo inhibicije. Drugače kot pri elementnem srebru so v primeru koloidnega srebra zaradi izredno majhnih velikosti delcev lahko protimikrobno delovali tako Ag^{+} kot Ag NPs, kar se je izrazilo v odlični protimikrobni učinkovitosti produkta.

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ranged from 100 to 500 nm and was far larger than the size of colloidal silver particles which did not exceed 20 nm. Bacterial reduction obtained by silver chloride and by colloidal silver was excellent, 99 to 100%, while in the case of nanosilver in the form of powder only 36% of bacterial reduction was obtained. Even after increasing the concentration of the latter by three times, the bacterial reduction reached the value of 60% only, which is unsatisfying. From these results, it was concluded, that antimicrobial activity of the studied agents did not depend only on the concentration and particle size, but also on the chemical form of silver. Namely, in the case of silver chloride, Ag^{+} which were released in the presence of water from the crystal of AgCl salt acted bactericidally. In the case of nanosilver in the form of powder antibacterial activity was also limited to the release of Ag^{+}, since nanoparticles of silver aggregated on the fibres to such an extent that they could not act antibacterially. Hence, silver nanoparticles can act bactericidally only if their size is smaller than 10 nm. Because large specific surface area of the particles is needed in order to release the Ag^{+} in the reaction of oxidation, the specific surface area of the silver aggregates was too small, so the concentration of released Ag^{+} did not reach the critical inhibitory concentration. Contrary to elemental silver, in colloidal silver Ag^{+} as well as silver nanoparticles acted antimicrobially, since the particle size was very small. This reflected in an excellent antimicrobial activity of the product.

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