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Silver Nanoparticles as a New Generation of Antimicrobial Prophylaxis

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*Over the last 20 years, metallic silver in the form of Ag nanoparticles has made a remarkable comeback as an example of a nanomaterial for control of microorganisms. The purpose of our study was a) to quantitatively estimate the antimicrobial effect of silver nanoparticles compared with that of silver ions and b) to check the efficacy of nanosilver as an antimicrobial agent against a range of microbes on the surface of water-soluble paint, 100% cotton fabric, and fibrous chemisorbent. Minimum inhibitory concentration tests quantitatively showed that Ag nanoparticles were less efficient than Ag⁺ ions against representatives of gram-positive / gram-negative bacteria and cosmopolitan saprotrophic fungi. Antifungal/antibacterial effects against *Aspergillus niger*, *Penicillium phoeniceum*, and *Staphylococcus aureus* were confirmed for nanosilver concentrations of even 1 µg/cm² on the surface of cotton fabric and 0.8 µg/cm² in water-soluble paint. As the concentration of nanosilver in water-soluble paint/cotton fabric was increased to 7 µg/cm², the growth of *Bacillus subtilis* and *Escherichia coli* was suppressed as well. Microbiological tests conducted over a period of 60 days showed that there was no biofilm growth on the surface of a silver nanoparticle-coated fiber sorbent during its everyday operation as a household water treatment filter. Thus, silver nanoparticles as an add-on to water-soluble paints, textile fabrics or fiber chemisorbents had a remarkable antibacterial/antifungal effect, although some of the Ag nanoparticles were agglomerated into larger colloidal clusters.*

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Наночастицы серебра – новое поколение антимикробной профилактики

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*В последние 20 лет наночастицы серебра (НС) нашли широкое применение в качестве антимикробного средства. Но вследствие недостаточной изученности воздействия наноматериалов на живые организмы вопрос эффективности и безопасности НС до сих пор остается открытым. В цели наших исследований входила количественная оценка антимикробной способности НС в сравнении с ионами серебра, а также оценка антимикробной эффективности НС при введении его в водорастворимые краски, хлопчатобумажные ткани и волокнистые сорбенты. Оценку проводили на основе стандартной методики определения минимальных ингибирующих концентраций. Результаты исследований для ряда грамположительных/граммотрицательных бактерий и сапротрофных грибов показали, что наночастицы серебра были менее эффективны по сравнению с ионами серебра. Тем не менее антигрибной и антибактериальный эффекты НС для культур *A. niger*, *P. rhoenicium* и *S. aureus* проявлялись уже при концентрации наносеребра 1 мкг/см² на поверхности хлопчатобумажной ткани и 0,8 мкг/см² в водорастворимой краске. Повышение концентрации наносеребра в водорастворимой краске/хлопчатобумажной ткани до 7 мкг/см² обеспечивало также подавление роста культур *B. subtilis* и *E. coli*. Было показано, что при 60-дневном систематическом использовании волокнистого сорбента, покрытого наночастицами серебра, в качестве бытового фильтра для очистки воды на нем не наблюдалось роста биопленок. Таким образом, наночастицы серебра в качестве добавки к водорастворимым краскам, текстильным тканям и волокнистым сорбентам обладают выраженным антибактериальным/противогрибковым эффектом, несмотря на наблюдающуюся тенденцию ряда наночастиц серебра к агрегации в коллоидные кластеры.*

Ключевые слова: серебро, наночастицы, наноматериал, волокнистый сорбент, водоподготовка.

Introduction

Metallic silver has been known for its antibacterial and antifungal properties. In ancient Greece and Rome, silver was widely used to control infections and spoilage. Since the 20th century, silver and its compounds have been used extensively in many bactericidal applications, including wound healing, water treatment, flower preservatives, etc. (Klasen, 2000). Over the last few decades, silver nanoparticles, defined as structures up to 100 nm in size, have been extensively investigated and found to have applications in catalysis (Lewis, 1993), optics (Murphy et al., 2005), electronics (Li et al., 2005), and other areas of science and technology (Niemeyer, 2001; Marambio-Jones and Hoek, 2010) due to their unique size-dependent optical, electrical, and magnetic properties (Oberdorster et al., 2005). Since 2000, most applications of silver nanoparticles have been related to their usage as antibacterial/antifungal agents (Morones et al., 2005; Buzea et al., 2007). Nanosilver application is one of the fastest growing product categories in the nanotechnology industry. For instance, there is a growing interest in the preparation of bactericidal cotton fibers containing silver nanoparticles for the textile industry (Lee and Jeong, 2004, 2005). Indeed, owing to their ability to retain moisture, ordinary cotton fabrics provide an excellent environment for microorganisms to thrive (Chen and Chiang, 2008). There is also a promising trend to impregnate commercially available paints with silver nanoparticles. For example, nanosilver-based wall paint would prevent the formation of mold inside buildings and the growth of algae on outside walls (Khaydarov et al., 2009a).

The lack of knowledge on the mechanism of the bactericidal effects of silver nanoparticles

in combination with the significant growth of applications of nanosilver in various branches of industry over recent years has caused new concerns that silver nanoparticles may have a toxic effect on human health (Lok et al., 2007; Khandani et al., 2017). In the 20th century, a popular belief was that except for causing argyria, silver was relatively nontoxic to mammalian cells (Chen and Schluesener, 2008). However, studies conducted in recent decades (Soto et al., 2005; Braydich-Stolle et al., 2005; Hussain et al., 2005; Grodzik and Sawosz, 2006) have shown that at the nanoscale, silver-based materials can exhibit significant toxicity to animal and human cells. These issues must be addressed before people rush to indulge into the nanosilver boom (Chen and Schluesener, 2008).

The purpose of our study was a) to quantitatively estimate the antimicrobial effect of silver nanoparticles compared with that of silver ions and b) to check the efficacy of nanosilver as an antimicrobial agent against a range of microbes on the surface of paints, 100% cotton fabrics, and fibrous chemisorbent.

Materials and methods

Preparation of Ag ions and nanoparticles

The process for producing silver nanoparticles was based on the use of an inexpensive two-electrode setup, in which the anode and the cathode are made from bulk Ag metal, which is to be transformed into Ag colloidal particles. We used two polished silver plates (85 mm x 20 mm x 4 mm) as the anode and the cathode, which were placed vertically face-to-face 10 mm apart. The electrodes were immersed in an electrochemical cell filled with 500 mL of distilled water obtained using an

ordinary, commercially available water distiller (DE-25, Russia). Electrolysis was performed in the temperature range of 20-95°C at a constant voltage of 20 V. The polarity of the direct current between the electrodes was changed every 30 – 300 sec, and intensive stirring during the process of electrolysis was used to inhibit the formation of precipitates. Silver nanoparticle solutions produced in this way were stored under ambient conditions in glass containers (Khaydarov et al., 2004). The water-based silver colloidal solution was obtained by our three-stage process based on the electroreduction of silver ions in water (Khaydarov et al., 2009). The nanosilver particles produced were spherical, with a diameter of 7 ± 3 nm (Fig. 1). The simplicity of this synthesis route allows low-cost fabrication of large amounts of long-lived silver nanoparticles. No chemical stabilizing agents are generally required, which expands the technological viability of the process and industrial applications of the method.

The concentrations of silver nanoparticles and ions in solutions were determined by neutron activation analysis (NAA) (Soete et al., 1972). Samples were irradiated in the nuclear reactor of the Institute of Nuclear Physics (Tashkent, Uzbekistan). The product of the nuclear reaction $^{109}\text{Ag}(n,\gamma) \rightarrow ^{110}\text{mAg}$ has a half-life ($T_{1/2}$) = 253 days.

The silver concentration was determined through measurements of the intensity of gamma radiation with an energy of 0.657 MeV and 0.884 MeV emitted by ^{110}mAg . A Ge(Li) detector with a resolution of approximately 1.9 keV at 1.33 MeV and a 6144-channel analyzer were used for recording gamma-ray quanta.

The morphology of silver nanoparticles on the surface of cotton and paint samples was observed by field emission scanning electron microscopy (FE-SEM; JSM-6700F, JEOL, Japan). The size and shape of the nanoparticles in solution were determined by transmission electron microscopy (TEM) (LEO-912-OMEGA, Carl Zeiss, Germany).

Antimicrobial activity assay

To evaluate the antibacterial and fungicidal properties of silver particles, *Escherichia coli* was used as a representative gram-negative bacterium; *Staphylococcus aureus* and *Bacillus subtilis* were used as gram-positive bacteria; and *Aspergillus niger*, *Aureobasidium pullulans* and *Penicillium phoeniceum* were used to represent cosmopolitan saprotrophic fungi. The minimum inhibitory concentrations (MICs) of solutions for various microbes were determined using the microdilution broth susceptibility test. Nutrient

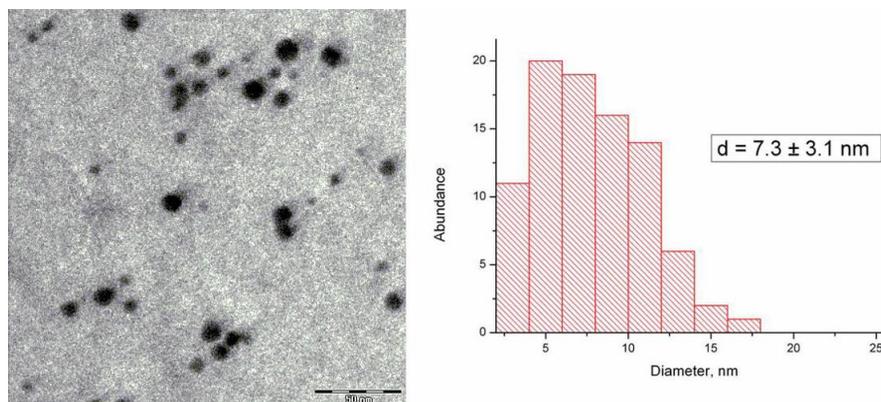


Fig. 1. A typical TEM micrograph (scale of 50 nm) and a size distribution histogram for silver nanoparticles prepared by the electrochemical method

broth used in the microdilution method contained peptic digest of animal tissue (50.00 g/L), beef extract (1.5 g/L), sodium chloride (5.00 g/L), and glucose (5 g/L) at pH 7.4 ± 0.2 . A standardized suspension of approximately 10^6 colony-forming units (CFU)/mL density was obtained by inoculating the culture in nutrient broth (Hi-Media) and incubating the tubes at 37°C for 3 h. Serial dilutions of the dispersion of Ag ions and the dispersion of silver nanoparticles were prepared within a desired range. Ten milliliters of the standardized culture suspension was then inoculated, and tubes were incubated at 37°C for 24 h. The MIC was defined as the lowest concentration of the inhibiting agent that completely inhibited bacterial growth, and the unit for the MIC was chosen as mg(Ag)/L. The MIC was examined visually by checking the turbidity of the tubes.

Antimicrobial finishing of cotton and paint samples

Common household acrylic paint widely used for renovating and decorating purposes was investigated. Silver nanoparticle-containing acrylic paint was obtained by diluting the initial paint sample with the Ag colloidal solution to achieve the desired nanosilver concentration within the desirable testing range of 2-50 ppm. For the antibacterial tests, a 22 mm x 22 mm pasteboard was covered with silver nanoparticle-containing acrylic paint.

Bleached woven cotton fabric weighing 98 g/m^2 was cut into equal-sized square pieces measuring 15 mm x 15 mm. The samples were immersed in a colloidal solution bath for 1 minute and squeezed thoroughly, with further drying at 60°C for 5 minutes.

To evaluate the antibacterial and fungicidal properties of Ag nanoparticles added to a cotton fabric and an acrylic paint, samples with different Ag nanoparticle contents, as well as control

samples, were immersed in a thin layer of beef-extract agar. One milliliter of a suspension with an approximately 10^5 CFU/mL density of the microorganisms to be tested was distributed uniformly on an agar surface and incubated at 28°C . Antimicrobial activity was evaluated according to the presence or absence of microbial growth just above the sample after a 24-h incubation for bacteria and a 72-h incubation for fungi. All microbiological tests were performed in triplicate.

Preparation of fiber sorbents

Polyacrylonitrile (PAN) cloth with a surface density of 1.0 kg/m^2 and thickness of 10 mm was used as the raw material for making ion-exchange sorbents. The cation-exchange fiber sorbents were obtained by treatment of PAN cloth with a 20% $\text{NH}_2\text{NH}_2\cdot\text{H}_2\text{O}$ solution at 70°C for 30 minutes and with a 5% solution of NaOH at $25\text{--}30^\circ\text{C}$ for one hour (Khaydarov and Khaydarov, 2006). We impregnated fibrous sorbents with our nanosized silver colloids by a method analogous to the technique used by Lee and Jeong (2005) for treating textile fabrics. The morphology of silver nanoparticles and silver-based sorbents was studied using TEM measurements. The concentration of silver nanoparticles was determined by neutron activation analysis (NAA) (Soete et al., 1972).

To evaluate the antibacterial and fungicidal properties of the sorbents modified with the nanosilver, we carried out the same microbiological procedures described above for cotton fabric samples.

Results and discussion

Minimum inhibitory concentration assays

The minimum inhibitory concentration (MIC) assays were conducted for electrically generated silver ions and silver nanoparticles using the gram-negative bacterium *E. coli*, gram-

positive bacteria *S. aureus* and *B. subtilis*, and fungus *P. phoeniceum* (see Table 1).

For an adequate interpretation of the results obtained, one needs to consider that in the case of silver nanoparticles, there is a combination of effects, including (i) that associated with Ag ions released from the nanoparticles and (ii) that of direct interaction of cells with nanoparticles (Wang et al., 2015). The purpose of our nanosilver MIC assay was not to reveal which of the effects prevails but was to quantitatively estimate the combined antimicrobial efficiency of nanosilver. Thus, to inhibit the growth of gram-positive and gram-negative bacteria and fungi (Table 1), it was necessary to apply higher concentrations of nanosilver than the concentration of electrically generated silver ions. Despite the pronounced antimicrobial effect, silver ions have only limited use as antimicrobial agents in applications such as medical products, clothing, and household articles. This fact is due to their rapid binding or inactivation by various substances present in a medium. This limitation can be overcome by using silver nanoparticles as an antimicrobial agent owing to the continuous release of Ag ions they provide (Kim et al., 2007).

Modified water-soluble paint

We impregnated water-soluble paints with our nanosized silver colloids. Most of the initial

silver nanoparticles agglomerated into clusters up to 200 nm in size because of attractive interaction forces between them (Fig. 2). The photographs in Fig. 3 show the growth of *A. pullulans*, *P. phoeniceum*, and *S. aureus* cultures on pasteboard samples modified by silver nanoparticles. The test results of the antibacterial and antifungal effects of nanosilver-based water-soluble paint are summarized in Table 2, where the plus sign indicates the occurrence of microbial growth, and the minus sign corresponds to the absence of visible growth.

An increase in the concentration of nanosilver in water-soluble paint to 5-6 $\mu\text{g}/\text{cm}^2$ led to the suppression of growth of *B. subtilis* and *E. coli*. Larger concentrations of nanosilver can potentially lead to undesirable color shifts in water-soluble paint with time. We observed an outside wall of a house painted (Tashkent, Uzbekistan) with nanosilver-modified (0.8 $\mu\text{g}/\text{cm}^2$) paint. The NAA tests conducted over a period of eight months (April-November, 2018) showed that no significant loss of silver nanoparticles in the wall paint occurred (Garipov et al., 2019).

We suggest that silver nanoparticles may be a commercially viable addition for use in the paint industry. Microbes can come into contact with walls in a number of ways, including by deposition of dust and fine aerosols, by human skin contact, and by splashes from liquids.

Table 1. Results of minimum inhibitory concentration (MIC) assays for silver nanoparticles and silver ions for some microbial species

Microbe species	Electrically generated silver ion MIC (mg(Ag)/L)	Silver nanoparticle (average particle size of 7 nm) MIC (mg(Ag)/L)
<i>Escherichia coli</i>	1	5
<i>Staphylococcus aureus</i>	0.6	3
<i>Penicillium phoeniceum</i>	0.5	2
<i>Bacillus subtilis</i>	2.4	29

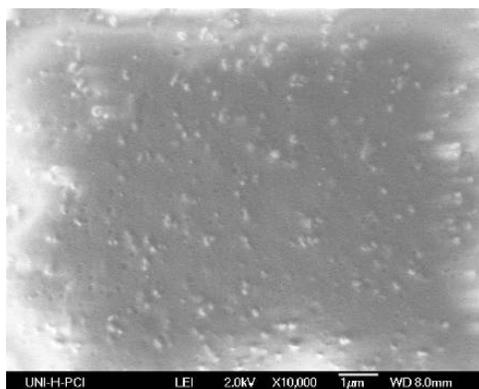


Fig. 2. A sample of water-soluble paint with immobilized silver nanoparticles



Fig. 3. Growth of *A. pullulans* (left), *P. phoeniceum* (middle), and *S. aureus* (right) cultures on pasteboard samples modified by silver nanoparticles (note, the white spots correspond to microbial colonies). Sample #1 is a control sample, i.e., it was covered with nonmodified paint; sample #2 was covered with the paint modified by silver nanoparticles at a density of 0.8 µg/cm²

Table 2. Antimicrobial effect of samples of water-soluble paint

Microbe species	Type of paint	
	Sample #1 (Nontreated paint)	Sample #2 (Nanosilver-modified paint, 0.8 µg/cm ²)
<i>Aspergillus niger</i>	+	-
<i>Penicillium phoeniceum</i>	+	-
<i>Aureobasidium pullulans</i>	+	-
<i>Staphylococcus aureus</i>	+	-
<i>Bacillus subtilis</i>	+	+
<i>Escherichia coli</i>	+	+
Control (samples on beef-extract agar)	+	-

Note: “+” – growth on beef-extract agar; “-” – absence of growth on beef-extract agar

Nanosilver-based wall paint can potentially prevent the formation of mold inside buildings and, by conjecture, the growth of algae on outside walls.

Modified cotton fabric

We also impregnated 100% cotton fabrics with nanosized silver colloids synthesized by the electrochemical technique (Khaydarov et

al., 2009). The particles had reasonably good dispersibility on the surface of a modified cotton spread. However, agglomeration of silver nanoparticles into larger clusters (up to 200 nm in size) was also observed (Fig. 4).

As shown previously (Khaydarov et al., 2009a, 2010), a cotton fabric with immobilized silver nanoparticles (mean particle size of 15 nm) with concentrations of 5 µg/cm², 3 µg/cm², and 1 µg/cm² can inhibit the growth of *A. niger*, *P. phoeniceum*, and *S. aureus* cultures, respectively, on beef extract agar. In the present study, we used an aqueous dispersion of 7±3 nm silver nanoparticles during the finishing process. This approach reduced the size of silver particles on the surface of the 100% cotton fabric from the micron size (as in our previous work) to ~100 nm. As a result, the antifungal/antibacterial effects

for *A. niger*, *P. phoeniceum*, and *S. aureus* were confirmed for nanosilver concentrations on the surface of the cotton fabric as low as even 1 µg/cm². The same experiments conducted for *E. coli*, *B. subtilis*, and *A. pullulans* cultures demonstrated a pronounced antimicrobial effect for concentrations of nanosilver of 7 µg/cm², 5 µg/cm², and 3 µg/cm², respectively.

To estimate the durability of the bacteriostasis effect of the silver-treated cotton fabric with respect to laundering, we compared the surface concentration of silver particles on fabrics before washing to the particles on them after 2, 5, and 10 washes (Table 3). The results indicated that silver particles were sufficiently bonded to the cotton fabric, which gave rise to good bacteriostasis effects after even 10 cycles of washing. These findings are encouraging results

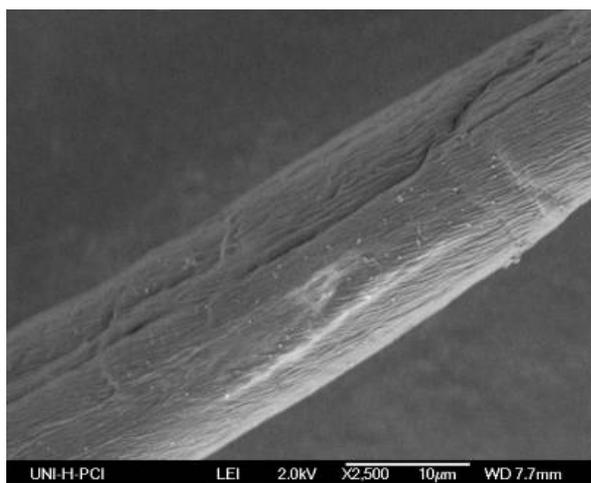


Fig. 4. A sample of 100% cotton with immobilized silver nanoparticles

Table 3. Laundering durability of silver nanoparticles on modified cotton fabric samples

	Ag concentration (µg/cm ²)
Before washing	5
After 2 cycles	3.5
After 5 cycles	1.8
After 10 cycles	1

suggesting that nanosilver-based textile materials give promise in various areas, for example, in the prevention of wound contamination with microorganisms, particularly fungi and bacteria, in hospitals.

Silver nanoparticle-coated fibrous sorbent for water treatment

Fibrous chemisorbents are widely used in the republics of the former USSR for treating industrial wastes to remove harmful substances, including heavy metals – lead, zinc, copper, iron and compounds containing chlorine, sulfate and chromate anions (Zverev, 2002). An advantage of the fibrous ion-exchange sorbents over resins is the high rate of the sorption process (approximately 100 times greater than that for resins) and small values of pressure drop of the sorbent layer for purified water (Khaydarov and Khaydarov, 2006).

Microorganisms can attach to the surface of fibrous sorbents during their operation as water treatment materials and grow into biofilms, i.e., the sorbents can act as a reservoir of pathogenic microorganisms and potentially can lead to the risk of infection. To prevent microbial growth and formation of a biofilm on the sorbents during their usage, we coated fibrous ion-exchange sorbents with silver nanoparticles. Nanosilver not

only attaches to the surface of the sorbent but also penetrates and associates under the surface due to the specific surface characteristics of the fibers, which are known (Zverev, 2002) to have a large surface area and high porosity (Fig. 5).

Most of the initial silver nanoparticles agglomerated into clusters of up to 300 nm because of attractive interaction forces between them. Our microbiological studies clearly demonstrated that a 20 mg/kg silver concentration provided a reliable prevention of the growth of these microorganisms on the surface of the fibrous ion-exchange sorbents (Khaydarov et al., 2019).

Microbiological tests conducted over the period of 60 days showed that there was no biofilm growth on the surface of a silver nanoparticle-coated fiber sorbent during its everyday operation as a household water treatment filter. NAA tests also indicated that nanosilver was sufficiently bonded to the fibers, as there was no significant loss of silver nanoparticles in the sorbent throughout that period (Khaydarov et al., 2019).

Conclusions

Ag nanoparticles synthesized using our 3-stage method based on electrochemical reduction of Ag⁺ ions in distilled water were proven to be very promising as a new generation

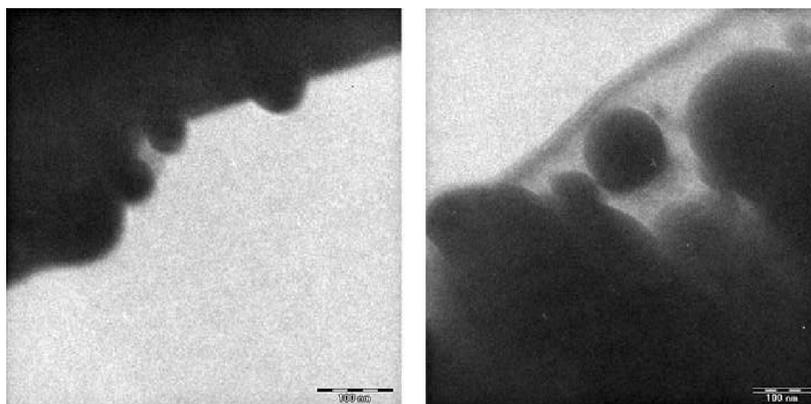


Fig. 5. A TEM image of silver nanoparticles on (left) and under (right) the surface of fibrous sorbent

of antimicrobial agents. Our minimum inhibitory concentration tests quantitatively showed that Ag nanoparticles were less efficient against representatives of gram-positive/gram-negative bacteria and cosmopolitan saprotrophic fungi than Ag⁺ ions. Because of the capability of Ag nanoparticles to continuously release Ag⁺ ions in a sufficient concentration, they are considered a better option than Ag⁺ ions in most antimicrobial applications. The tests carried out in the present study revealed that the obtained Ag nanoparticles as an add-on to water-soluble paints, textile fabrics or fiber chemisorbents had a remarkable antibacterial/antifungal effect, although some of the Ag nanoparticles were agglomerated into larger colloidal clusters.

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