Antimicrobial treatment based on green silver nanoparticles applied to textile heritage

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MIHAELA-CRISTINA LITE
RODICA ROXANA CONSTANTINESCU
NICOLETA BADEA
LAURA CHIRILĂ
DOINA TOMA
DEMETRA SIMION
ALINA POPESCU

ABSTRACT – REZUMAT

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Nanomaterials science expanded its application in various directions (biomedical, smart materials, sensors, optoelectronics, etc.). Silver nanoparticles (AgNPs) have been intensively tested within the textile department and demonstrated antimicrobial activity when applied under various forms (commercial colloidal nano-silver, in situ synthesis, etc.). The application of silver nanoparticles for historical artefacts conservation has been addressed, and several papers have reported the use of such treatments for cultural heritage conservation. In this work, the historical sample tested consisted of a cotton fabric, part of a priest's costume, dating back to the 18th century, provided by the Church of Saint Nicolae "Dintr-o Zi", located in Bucharest. The piece was treated with AgNPs-based treatment, which was phyto-synthesised by using a plant aqueous extract. The effect of the treatment on the sample was examined by studying fibres of the fabrics with electronic microscopy (SEM) and by quantifying the modification of the sample appearance, using chromatic parameters measurements. The antimicrobial activity was tested against gram-positive and gram-negative bacteria strains, Escherichia coli and Staphylococcus aureus, and the fungal strain Penicillium hirsutum. The layer of silver nanoparticles was uniformly distributed on the surface of the historical textile fibres. The changes in the appearance of the textile were minimal, with a total colour difference of 2.11. Evaluating the antimicrobial activity, superior antifungal performance was observed against the Penicillium hirsutum strain (for which the inhibition zone value was 12.5 mm). The FTIR spectrum demonstrated that the integrity of the textile fibre is maintained following the application of the treatment.

Keywords: *silver nanoparticles, conservation treatment, historical textiles*

Tratament antimicrobian pe bază de nanoparticule de argint „green” aplicate pe materiale textile de patrimoniu

Știința nanomaterialelor și-a extins aplicația în diverse direcții (biomedicală, materiale inteligente, senzori, optoelectronice etc.). În cadrul departamentului de textile, nanoparticulele de argint (AgNPs) au fost testate intens și au demonstrat activitate antimicrobiană, atunci când sunt aplicate sub diferite forme (nano-argint coloidal comercial, prin sinteză in situ etc.). Aplicarea nanoparticulelor de argint pentru conservarea artefactelor istorice a fost abordată și mai multe lucrări au raportat utilizarea unor astfel de tratamente pentru conservarea patrimoniului cultural. În această lucrare, proba istorică testată a constat dintr-o țesătură de bumbac, parte dintr-un costum de preot, datând din secolul al XVIII-lea, furnizată de Biserica Sfântul Nicolae „Dintr-o Zi”, situată în București. Piesa a fost tratată cu tratament pe bază de AgNPs, care a fost fito-sintetizat prin utilizarea unui extract apos de plantă. Efectul tratamentului asupra probei a fost examinat prin studierea fibrelor textile cu microscopie electronică (SEM) și prin cuantificarea modificării aspectului probei, folosind măsurători ale parametrilor cromatici. Activitatea antimicrobiană a fost testată împotriva tulpinilor de bacterii gram-pozitive și gram-negative, Escherichia coli și Staphylococcus aureus, și împotriva tulpinii fungice Penicillium hirsutum. Stratul de nanoparticule de argint a fost distribuit uniform pe suprafața fibrelor textile istorice. Modificările în aspectul materialului textil au fost minime, cu o diferență totală de culoare de 2,11. Evaluând activitatea antimicrobiană, s-au observat performanțe antifungice superioare față de tulpina Penicillium hirsutum (pentru care valoarea zonei de inhibiție a fost de 12,5 mm). Spectrul FTIR a demonstrat că integritatea fibrei textile se menține în urma aplicării tratamentului.

Cuvinte-cheie: *nanoparticule de argint, tratament de conservare, textile istoice*

INTRODUCTION

Many studies carried out in applied nanomaterials science have been highlighting the effectiveness of silver nanoparticles (AgNPs) against microbial contamination [1]. In the past decades, these nanomaterials have been tested for various applications, within the textile department, such as smart textiles [2], wound dressings [3], medical textiles [4], textile cultural heritage [5], etc. The papers reporting the antimicrobial effectiveness of AgNPs-based treatments applied on textiles paved the way towards using the AgNPs in conservation science [6]. Since the growth of microorganisms and their metabolic products constitute one of the main causes of fibre degradation, the solution of using an antimicrobial treatment represents a suitable approach [7, 8]. Moreover, the advantage of using such treatments lies in a change of the conservation strategy, by approaching a biocidal effect rather than bacteriostatic, conferred by the conventional methods (appropriate microclimate or treatments designed to create an unfriendly environment for the growth of microorganisms) [9, 10]. Furthermore, when synthesising AgNPs by environment-friendly methods, the costs are reduced, the manipulation of the treatments does not endanger people or animals, and there is no hazardous impact on the environment [11, 12]. The clean manufacturing character of AgNPs represents a crucial benefit, since it does not involve complex procedures, toxic compounds or and does not produce residues [13]. The potential limitation might include the reproducibility, especially scaling up the production of *green* AgNPs, since the phyto-content of plant extracts might differ depending on the conditions in which the plant was cultivated. Many researchers reported the production of *green* AgNPs, exhibiting antimicrobial properties, using extracts of different parts of the plant, leaf [14], seeds [15] flower [16], root [17] fruit [18] etc. Nivedhitha Kabeerdass studied the biomedical and textile applications of AgNPs fabricated by plant-mediated synthesis. The antimicrobial test on cotton fabrics provided values of the inhibition zone in the range 6–10 mm against *E. coli*, *S. aureus*, *P. aeruginosa*, *K. pneumoniae*, *K. oxytoca*, and *A. baumanii*, respectively [19]. Nagah S. Saada and the collaborators studied the effect of *green* AgNPs against the biodegradation of historical parchment [20]. Similarly, Amr Fouda and her group conducted tests regarding the effect of biosynthesised AgNPs applied to a historical book and evaluated their performance against fungal deterioration [21].

In the present research, the performances of a phyto-synthesised AgNPs-based treatment were tested on a historical sample. The morphology of the sample fibres was evaluated using the microscopic technique. Moreover, the appearance of the sample has been rigorously verified by measuring the chromatic parameters before and after applying the AgNPs dispersion. This aspect is of great importance in the field of conservation-restoration [22,23]. The IR spectra were recorded to examine possible changes in the fibre structure.

The antimicrobial tests focused on evaluating the effect against a gram-negative strain (*Escherichia coli*), a gram-positive strain (*Staphylococcus aureus*), and a fungal strain (*Penicillium hirsutum*).

EXPERIMENTAL

Materials and methods

All the regents used were purchased from Merck. The AgNPs dispersion was phyto-synthesised and applied according to the protocol reported in our previous research [24]. The ratio of extract: silver precursor was 1:3 (v/v). The sample used to test the effectiveness of the proposed treatment, based on *green* AgNPs, was collected from a historical artifact, dating back to the 18th century, and was provided by the Church of Saint Nicolae "Dintr-o Zi", located in Bucharest, founded in 1702, by the wife of ruler Constantin Brâncoveanu. This historical sample, made of cotton, is part of a priestly costume.

The antimicrobial tests were conducted using the bacteria strains *Escherichia coli* ATCC 10536 and *Staphylococcus aureus* ATCC 6538, and the fungal strain *Penicillium hirsutum* ATCC 52323. For the culture media, Casein Soya Bean, TSB Tryptic Soy Broth, NB Nutrient broth, EA Enumeration Agar, Digest Agar, SCDLP Casein Soya Bean Digest, and PDA, Potato-Dextrose-Agar were used.

The antibacterial tests were carried out by the diffusion method in agar medium (inhibition zone method) [25, 26]. The entire surface of the Petri dishes was inoculated with the same volume of sample from each microorganism strain, and textile samples (10 mm diameter) were placed on the surface of the nutrient medium and then incubated at 37°C for 24 hours. The formation of a clear inhibition zone (IZ) indicates the antimicrobial efficiency of the samples. The zone of inhibition is calculated according to the following formula:

$$IZ = \frac{D - d}{2} \quad (1)$$

where D is the total diameter of the sample and zone of inhibition (mm), and d is the diameter of the sample (mm).

For the antifungal analysis, the culture medium is autoclaved, then poured into Petri dishes. In a test tube with the strain to be tested, add 10 mL of sterile water and scrape, then take 1 ml of the suspension with which the entire surface of the Petri dish is seeded. The evolution/involution of the pathogen culture was followed at 72–96 hours by comparison with the negative control plate.

Characterization techniques

The performance evaluation of the applied treatment was quantified by analysing AgNPs deposits on textile fibres, using scanning electron microscopy (SEM), FTIR spectroscopy, chromatic analysis and by determining the antimicrobial properties.

For performing SEM analysis, the FEI Quanta 200 instrument (ThermoFisher Scientific, Waltham, Massachusetts, USA), equipped with an Everhart-Thornley (ET) detector, was used at an accelerating voltage of 15 kV.

The IR spectra were recorded using an FT-IR-ATR spectroscopy instrument from ThermoFisher, and the spectral range was 4000-400 cm^{-1}.

The Datacolor (D65/10 lamp) instrument (Datacolor, Inc., Lucerne, Switzerland) was used for measuring the chromatic parameters, and they were expressed in the CIE L*a*b* system.

RESULTS AND DISCUSSION

Figure 1 presents the image of the heritage sample before and after treating it with the AgNPs dispersion.

Evaluation of the morphology of heritage textile fibres treated with AgNPs dispersion

The morphology of the sample fibres, before and after applying the treatment, is presented in figure 2. The image shows that silver nanoparticles are uniformly distributed on the surface of the fibres.

Chromatic parameters of the heritage textile treated with AgNPs dispersion

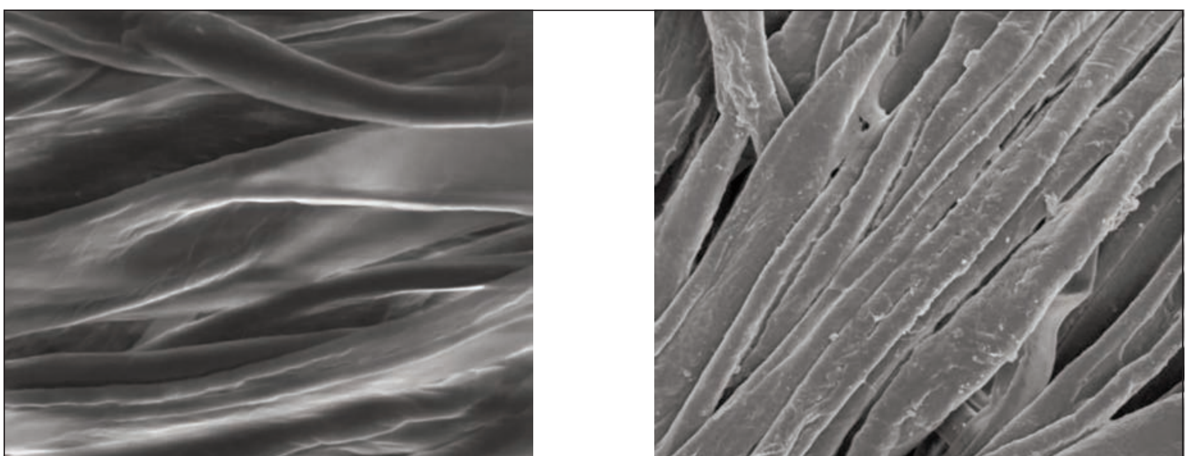
The yellowing of textiles is the most visible indicator of their degradation [27]. In the present case, the historical sample shows shades of yellow, quantified by the value of the parameter b* = 15.50 and a brightness (L*) of 85.52 (table 1). Following the application of AgNPs dispersion, it is noticed that no colour changes occur (figure 3). Moreover, the parameter b* decreased from 15.50 to 14.12.

The colour difference recorded for the textile sample following the application of AgNPs dispersion fulfils the criteria for conservation-restoration of heritage objects [28]. Artefact appearance changes are minimal, and the time patina was maintained following the application of the AgNPs dispersion.

The long term-performance of the AgNPs dispersion is expected to have a minimum effect the appearance of the historical materials, as it resulted from a previous study on the chromatic changes that occurred following the exposure of textiles treated with AgNPs dispersion to conditions of accelerated aging,

a *b*

Fig. 1. Image of the heritage sample: *a* – before; *b* – after treating it with the AgNPs dispersion



a *b*

Fig. 2. SEM images of the historical textile sample: *a* – before; *b* – after applying the AgNPs-based treatment

Table 1

CHROMATIC PARAMETERS OF THE HISTORICAL SAMPLE BEFORE AND AFTER APPLYING THE AGNPS-BASED TREATMENT

Chromatic parameter	L*	a*	b*	ΔL*	Δa*	Δb*	ΔE*
Untreated samples	85.52	1.92	15.50	-	-	-	-
Samples treated with AgNPs	84.22	2.85	14.12	–1.30	0.93	–1.38	**2.11**

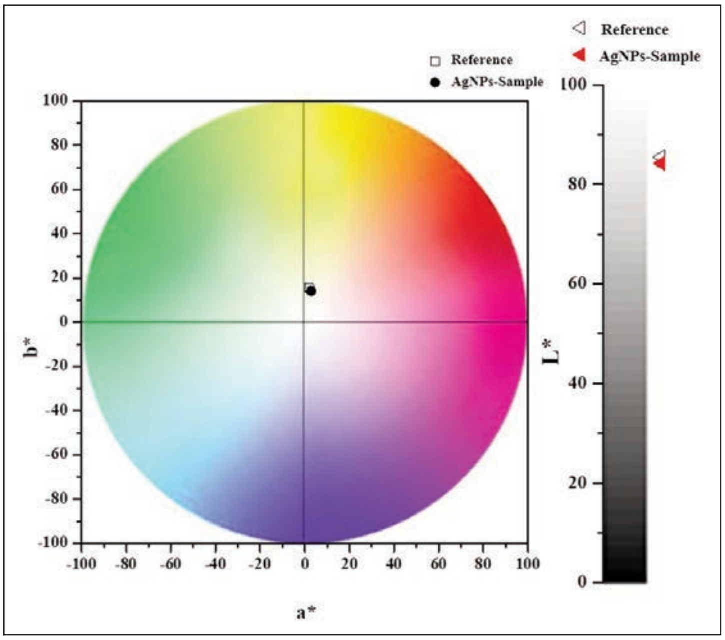


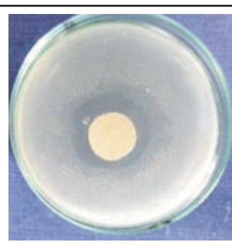
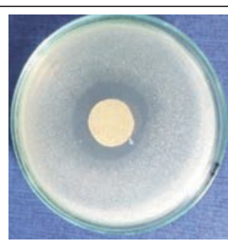
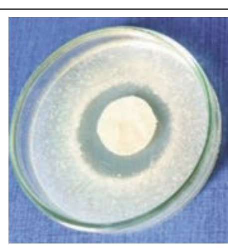
Fig. 3. Chromatic diagram of a historical sample treated with AgNPs dispersion synthesised with *Stellaria media* extract

through a forced degradation process involving UV light, temperature and humidity [29].

The antimicrobial effect of AgNPs dispersion applied to the heritage textile samples

Antimicrobial tests were performed on the bacterial strains of *Escherichia coli*, *Staphylococcus aureus*

Table 2

IMAGES OF PETRI DISHES INOCULATED WITH THE TESTED MICROBIAL STRAINS AND INCUBATED WITH THE HISTORICAL TEXTILE SAMPLE TREATED WITH THE AGNPS DISPERSION	
Microbial strain	**AgNPs-treated samples**
Escherichia coli	
Staphylococcus aureus	
Penicillium hirsutum	

and the fungal strain of *Penicillium hirsutum* (table 2). It is observed that the AgNPs dispersion exhibits superior antimicrobial activity against the tested fungal strain. The sensitivity of the pathogen to the antimicrobial action of AgNPs increased in the order *Escherichia coli* < *Staphylococcus aureus* < *Penicillium hirsutum* (figure 4).

The cytotoxic effect of AgNPs on microorganisms could be attributed to the generation of intracellular reactive oxygen species (ROS) in highly reactive bacteria. The interaction of AgNPs with microbial cells often generates ROS radicals, superoxide (O_2•$^-$), hydroxyl radical (OH•), and hydrogen peroxide (H_2O_2), which can interact and inactivate various cellular components such as DNA, cell membrane or enzymes, leading to the death of bacteria. Also, another factor favouring the destruction of cells is the zeta potential of Ag nanoparticles, which causes disturbances in the lipid bilayers of the cell membrane, leading to the loss of ions and other components, the formation of pores, as well as the dissipation of the electric charge of the membrane [30]. Although the exact mechanism of action of AgNPs on microbial agents is still unknown, the possible mechanism involves the attachment of nanoparticles to the outer cell membrane and its rupture, followed by penetration into the inner membrane and inactivation of respiratory chain dehydrogenases.

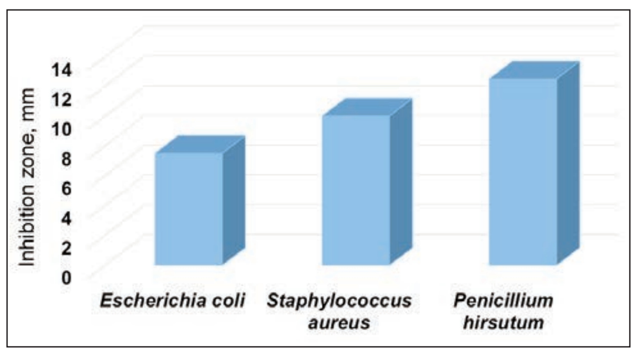


Fig. 4. The zone of inhibition formed on the *Petri dishes inoculated with the tested microbial strains and incubated with the historical textile sample treated with the AgNPs dispersion*

Characterisation by IR absorption spectroscopy of the historical textile sample treated with AgNPs dispersion

The effect of the AgNPs dispersion on the chemical composition of the historical sample was assessed by IR absorption spectroscopy. Figure 5 shows the overlapped FT-IR spectra recorded for the untreated and treated sample, respectively, which represents the fingerprint of the chemical bonds present in the sample. The bands present in the FTIR spectra are characteristic of cotton and are due to the cellulose macromolecule. These bands appear at 3273 cm^{-1},

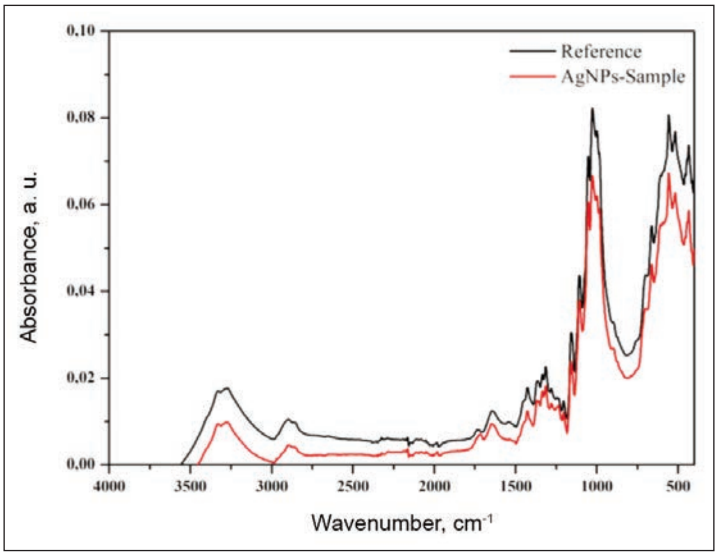


Fig. 5. Absorption IR spectra of the historical textile before and after applying the AgNPs dispersion

attributed to O–H bond stretching energy, 2899 cm^{-1}, corresponding to C–H bond stretching, 1426 cm^{-1}, attributed to C–H bond vibration, 1314 cm^{-1}, bond torsion C–H and 1027 cm^{-1}, C–O bond stretching. Spectra obtained for the sample treated with AgNPs reveal no new bands compared to the control, demonstrating that no chemical reaction occurs on the cellulose during AgNPs coating [31].

CONCLUSIONS

The silver nanoparticles adhered uniformly and abundantly to the textile fibres belonging to the historical sample. After depositing the AgNPs dispersion, changes in the appearance of the textile were minimal, with a total colour difference of 2.11, while maintaining the patina of time. Evaluating the antimicrobial activity, superior antifungal performance was observed against the *Penicillium hirsutum* strain (for which the inhibition zone value was 12.5 mm). The FTIR spectrum demonstrated that the textile fibre is not affected at the chemical composition level, maintaining its integrity following the application of the treatment.

The limitation of such treatments might involve the potential toxicity of AgNPs in mammalian cells. However, given the external use and the formulation of the treatment into dispersions, their manipulation should have no impact on human health. Nevertheless, this study can be further pursued to evaluate the durability of the treatments or the potential resistance that the bacteria might acquire over time. Future experiments aim to study the durability of the produced dispersion in the context of antimicrobial performances, pursuing antimicrobial testing on a wider spectrum of bacterial strains and different types of textile materials.

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Authors:

MIHAELA-CRISTINA LITE[1,2], RODICA ROXANA CONSTANTINESCU[1], NICOLETA BADEA[2], LAURA CHIRILĂ[1], DOINA TOMA[1], DEMETRA SIMION[1], ALINA POPESCU[1]

[1]National Research and Development Institute for Textiles and Leather – INCDTP,
Lucretiu Patrascanu, 16, 030508 Bucharest, Romania
e-mail: office@incdtp.ro

[2]Politehnica University of Bucharest, 1-7 Gheorghe Polizu Street, 011061, Bucharest, Romania
e-mail: secretariat@chimie.upb.ro

Corresponding author:

MIHAELA CRISTINA LITE
e-mail: cristina.lite@incdtp.ro