Biopolymers from Composted Biowaste as Stabilizers for the Synthesis of Spherical and Homogeneously Sized Silver Nanoparticles for Textile Applications on Natural Fibers

UNIVERSITÀ DEGLI STUDI DI TORINO

Biopolymers from composted biowaste as stabilizers for the synthesis of spherical and homogeneously sized silver nanoparticles for textile applications on natural fibers

Roberto Nisticò[a,*], Marco Barrasso[a], Galo Antonio Carrillo Le Roux[c], Marcelo Martins Seckler[c], Walter Sousa[c], Mery Malandrino[a], Giuliana Magnacca[a,b]

[a] University of Torino, Department of Chemistry, Via P. Giuria 7, 10125 Torino, Italy
[b] NIS Centre and INSTM Reference Centre, Via P. Giuria 7, 10125 Torino, Italy
[c] Escola Politecnica, Universidade de Sao Paulo Departamento de Engenharia Quimica, Av. Prof. Luciano Gualberto, trav. 3, 380, Sao Paulo SP, Brazil
* Corresponding author. E-mail: roberto.nistico@unito.it

Abstract
The use of bio-based substances (BBS) obtained from composted biowaste as stabilizers for the production of silver nanoparticles (AgNPs) in substitution to citrate is investigated herein, evaluating the functionalization of natural fibers for textile antibacterial applications. The results obtained evidenced that BBS can substitute citrate as reducing/stabilizing agent in the synthesis, inducing a geometrical control (in shape and size) of the AgNPs. Two different substrates were selected (wool and cotton) and two dip-coating deposition techniques investigated. The release of AgNPs from the supports in water was evaluated under two different experimental conditions: 1) soaking (static conditions) for 7 and 15 days, simulating the contact with sweat, and 2) centrifugation (dynamic conditions), simulating a washing machine treatment. A wide physicochemical characterization was carried out to evaluate the effects of BBS on the morphology and stability of AgNPs suspensions as well as the functionalization effectiveness.

Keywords: biopolymers; cotton; nanoparticles; silver; wool.

1. Introduction
Noble metals (i.e. silver and gold) are conventionally well-known antimicrobial materials.[1–6] Going back in time, for example in abdominal surgery, silver was the first material utilized for sutures by ancient Greek and Romans since well tolerated by the human body.[7] However, these early prostheses presented lots of clinical problems. In fact, the use of silver filigrees comported patient discomfort (caused by rigidity) and stimulated fibrous reactions in human tissues because of their fragility. Nowadays, the interest around the antimicrobial properties of silver has blossomed again.[8] Silver nanoparticles (AgNPs in the following), with diameter sizes between 1 to 100 nm, are topic of research in nanotechnology for clinical application[9, 10] since their shape and size affect the antibacterial capacity and their use in wound application (or silver care) has become the subject of studies by many researchers in the multidisciplinary biomedical field.[11]
Moreover, with a rising interest in personal health and hygiene, textiles with antimicrobial properties are becoming increasingly a desirable aim by textile manufacturers.[12] In fact, recently the surface modification of commercial textiles by AgNPs loaded/grafted received great attention and many studies were focused on natural fibers functionalization (mainly, wool and cotton).[4,12–20]
The standard procedure for obtaining AgNPs is a chemical reduction of Ag^{I} salts into metallic Ag^{0}, which easily aggregate forming metallic clusters.[21–23] This is made by using appropriate reducing agents which act also as colloidal suspension stabilizers (usually trisodium citrate, sodium borohydride, glucose, chitosan, hydrazine). Historically, the most diffused procedure for the synthesis of AgNPs is the Turkevich process,[24] which consists in the reduction of silver nitrate in aqueous environment at 80–90 °C by trisodium citrate allowing to produce 20–200 nm sized AgNPs.

Such citrate-induced reactions have been widely studied, but some aspects are still unclear.[25–26] Basically, the synthesis consists of five conceptual steps: 1) oxidation of citrate forming dicarboxyacetone, CO_2, H^+ and two electrons; 2) reduction of Ag^+ into Ag^0 by citrate-derived electrons; 3) formation of metallic clusters Ag_x^{n+}, positively charged; 4) cluster complexation by citrate molecules (stabilizers) and clusters growing until reaching a stable dimension; 5) growing of the AgNPs thus formed by Ostwald maturing. Several factors can influence these steps (and therefore the AgNPs final shape and size), such as temperature, pH, reaction time, and citrate concentration.[27]

The citrate route, however, presents some limitations due to the experimental conditions. For instance, by working at low citrate concentrations (or prolonged reaction time), it is not possible to control the coalescence of the particles, their aggregation and formation of dendritic structures with dimensions dramatically higher than the desired ones. Nevertheless, as reported in the literature,[28] different citrate ionic species can be obtained by varying the pH, thus influencing the AgNPs final size. Therefore, even though the Turkevich process via citrate is a very simple, safe and widely-used method, for a better sizing and morphologically-controlled synthesis it is necessary to use other procedures with more effective stabilizers.

As widely discussed in the literature, the valorization of urban and/or agricultural biowastes into chemicals (re-entering them into the economic cycle) is becoming an increasingly important issue, which has caught the attention of experts worldwide.[29] In particular, the use of substances derived from agricultural biowaste as alternative to the classical citrate and $NaBH_4$ for obtaining metallic NPs (mostly AgNPs) is a very interesting field of research which involved researchers from all parts of the world. As it has been reported in the literature, flavonoids and other biological molecules extracted from pomegranate or lemon peels (i.e. Kaempferol and its derivatives),[30, 31] leaf extracts from Artemisia nilagirica,[32] and Aloe vera[33] were investigated as greener reducing/stabilizing agents for the AgNPs production. Additionally, the preparation of lignin-stabilized AgNPs was also studied, although the procedure adopted required the use of microwave irradiation.[34] In this context, bio-based substances (BBS-GC, where GC stands for green compost) isolated from urban biowastes are biological macromolecules with a complex lignin-derived structure containing several functionalities (namely, acid and basic functional groups bonded to aromatic and aliphatic chains, the BBS-GC chemical structure is fully reported in Table S1 of the Supporting Information, SI).[35, 36] The polymeric nature of these BBS suggests their potential use as stabilizing agents for greener synthesis of nanoparticles.[37] All these substances show high amounts of O-containing groups (namely carboxylic acids) that can develop the same function as Turkevich's citrate. In fact, the oxidation of carboxylic acids should produce ketonic species (as dicarboxyacetone for citrate), thus giving the electrons necessary for the reduction of Ag^+ into Ag^0. Additionally, the formation of metallic clusters Ag_x^{n+}, positively charged can be easily complexed by COOH groups of these products guaranteeing a high dispersion of the forming particles.

Therefore, the aim of this study is the use of BBS as synthesis intermediates/stabilizers for the production of AgNPs. A comparison between the standard citrate and BBS routes has been realized. AgNPs produced by these methods were loaded upon the surface of natural fibers (i.e. cotton and wool) for antibacterial (i.e. odorless) textiles application. Moreover, two different dip-coating deposition techniques were investigated: 1) cold deposition (by immersing natural fibers into a previously prepared AgNPs aqueous suspension), 2) hot deposition (by immersing natural fibers into a $AgNO_3$ aqueous solution and, subsequently, promoting the in situ reduction of silver ions in the presence of the fibers). The AgNPs functionalized fibers were physicochemically characterized before and after static soaking in water for 7 and 15 days to simulate the contact with sweat, and after centrifugation in order to evaluate the effect of washing machine treatment. Also the Ag release in water after each one of the three treatments was determined.

2. Results and discussion

2.1 Synthesis and characterization of the AgNPs colloidal suspensions

The modified Turkevich procedure, using citrate and/or BBS as Ag reducing agent/stabilizer, induced the formation of a colloidal suspension characterized by nanoparticles with different shapes and sizes. The morphology and size of the AgNPs is deeply influenced by the experimental conditions (i.e. temperature and time of reaction, stirring, concentration and nature of reducing agents/stabilizers). To verify the capacity of BBS as both reducing agent and stabilizer, several experiments were performed by partially and/or totally replacing citrate (standard reference) with BBS. In particular, four different reaction mixtures were investigated, partially substituting the amount of citrate (expressed in wt.%) with BBS: 1) 0% BBS (AgNPs_0), 2) 50% BBS (AgNPs_50), 3) 75% BBS (AgNPs_75), and 4) 100% BBS (AgNPs_100).

Using the classical citrate route, it was possible to follow the progress of AgNP formation by directly looking at the solutions due to the color change induced by the well-known Ag plasmonic resonance (the solution turns yellow). On the other hand, for BBS-containing suspensions, the direct visual observation was not possible because the brownish color of the BBS covered all the changes of the suspension. Only UV/Vis spectroscopy allowed a clear observation of the AgNP formation after subtraction of the Ag-free reaction-mixture spectrum.

As it can be observed in Figure 1, AgNPs synthesized in the presence of citrate (AgNPs_0) present a maximum of absorbance at 440 nm, in agreement with a nanoparticle size of 30–50 nm, as reported in the literature.[38] Nevertheless, the very broad and complex peak shape indicates a certain degree of polydispersion in terms of AgNPs shape and size, indicating the additional presence of larger particles evidenced by the tail at wavelength higher than 440 nm, probably formed by coalescence phenomena, also caused by suspensions aging.[26]

Since the UV/Vis signals of BBS-stabilized AgNPs were very intense, all the suspensions were diluted 1:10. In these cases, a narrower, symmetric, blue-shifted signal due to the plasmonic resonance of AgNPs was observed (the maximum of absorbance in this case is at 420 nm). This suggests that BBS allow the synthesis of AgNPs smaller and homogeneously distributed in size than citrate.

Figure 2 shows TEM micrographs of the AgNPs suspensions synthesized in the presence of different amounts of BBS. The suspensions were characterized just one day after the synthesis to avoid aging-induced coalescence effects. These micrographs confirmed the previous results. In particular, by using citrate as stabilizer (Figure 2A,B), it was possible to see elongated prism-shaped nanoparticles 100–150 nm long with hexagonal section whose diagonals correspond to about 10–50 nm and high polydispersity (average diagonal is 35.9±9.2 nm). The introduction of BBS in the formulation causes a modification in AgNP shape and size. In fact, AgNPs_50 (Figure 2C,D) evidenced a sharper polydispersity of nanoparticles surrounded by a thin film of amorphous, organic matter due to BBS residues. By increasing the BBS content (AgNPs_75, Figure 2E,F), AgNPs appear smaller, but still some large particles are visible. All the particles are spherical or almost spherical, with sizes mainly in the range between 10 and 30 nm. Finally, micrographs of AgNPs_100 suspension (100% BBS, Figure 2G,H) clearly show the formation of small well-dispersed spherical nanoparticles whose average diameter corresponds to about 10 nm.[34] The nanoparticle size distributions of the four AgNPs suspensions are reported in Figure 3: each histogram represents the statistical population of AgNPs with the size reported in the abscissa axis. The figure shows that the higher the BBS amount used in the synthesis, the smaller the nanoparticles sizes. Ag dendritic structures were observed for AgNPs_0 and only rarely for AgNPs_100 samples, indicating again that BBS acts as AgNPs stabilizer and size-controlling agent (see Figure S1). Probably, this phenomenon could be explained as a consequence of the high steric hindrance of BBS and by its hyperbranched chemical structure that limit the clusters growing step.

2.2. Aging of the AgNPs colloidal suspensions

To evaluate possible aging, the AgNPs suspensions (namely AgNPs_0 and AgNPs_100) were kept in the dark at RT from one day to six months and dimensionally monitored by means of UV/Vis

spectroscopy and TEM analysis. Also the AgNP size distribution was evaluated after one day and six months of aging.
Figure S2 shows the macroscopic aspect of an AgNPs_0 suspension as a function of the aging time. In particular, due to coalescence, citrate-stabilized AgNPs colloidal suspensions move from yellow to grey, indicating an increase of the particles' dimension. The greyish aspect is characteristic of suspensions of Ag particles in the micrometric size range. Analogous considerations on the macroscopic aspect cannot be made for BBS-stabilized suspensions due to the biopolymer brownish color, which remains unaltered over time.
The dimensional characterization of the particles after six months of aging was carried out for both citrate- and BBS-stabilized samples and the results are reported in Figure 4, compared with the size distribution relative to the fresh samples.
In both cases, an increase of the average dimensions was observed, but for the AgNPs_100 sample, the size of the particles, although characterized by a larger distribution with respect to the starting one, increases in a more limited extent than in the case of AgNPs_0. Accordingly to this behavior, the UV/Vis spectra of AgNPs_100 remain almost unchanged after 50 days of aging whereas a clear red-shift of the band is observable after only 15 days of aging in the case of AgNPs_0. Moreover, dendritic structures were evidenced in the TEM micrographs of the latter sample. These results suggest that citrate (Turkevich reference) presents a very limited AgNPs stabilizing effect in time with respect to BBS.

2.3. AgNPs loading on the natural fibers and physicochemical characterization

Both fibers, wool and cotton, were coated following the cold and hot deposition methods assisted by citrate and BBS. A first indication of the efficacy of AgNPs loading was the color assumed by the functionalized fibers, observable in Figures S3 and S4 compared with the unfunctionalized reference fibers reported in Figure S5. The loading of AgNPs_0 by cold deposition on wool induced a beige color to fibers, leaving the loading bath still colored. The loading of AgNPs_0 by hot deposition induced a light yellow color to wool fibers, leaving the loading bath uncolored. Vice versa, the loading of BBS-stabilized AgNPs (AgNPs_100) by using both procedures (i.e. cold and hot deposition) induced a light brown color to fibers, leaving the loading bath still colored. In general, the coating on the wool fibers was homogeneous for both deposition methods, as witnessed by the coloring quality.
AgNPs_0 loading on cotton fibers showed almost the same behavior as that described for wool fibers, thus a beige color for cold deposition and a light-yellow color for hot deposition. AgNPs_100 loading gave a very inhomogeneous brown color, broad contribution in the range 200–800 °C, with a maximum rate at about 300 °C due to the wool thermal degradation leaving a carbonaceous residue of about 25 wt. %.[39] As evidenced in the differential thermal profile, the second thermal phenomenon comprehends two main contributions explained in the literature as due to a differential melting process of orto- and para- cortical cells or to melting/degradative phenomena.[40–42] No significant modifications of this trend were evidenced after all the AgNPs functionalization methods investigated.
The TGA curves obtained for the cotton fibers are collected in Figure 5B. According to the literature,[43] two weight losses were evidenced: a first one, limited to 7 wt. %, due to the elimination of water molecules present on the fibers surface at about 100 °C, and a second remarkable one due to the cotton thermal degradation in the range 300–350°C, leaving a carbonaceous residue of about 12 wt. %. Also in this case, no significant modification of this trend was evidenced after all the AgNPs functionalization methods investigated.
The infrared spectra of natural fibers before and after functionalization are collected in Figure 6. For the sake of brevity, only the hot deposition of BBS-stabilized AgNPs was considered, since similar results were obtained for all the deposition methods investigated. The FTIR/ATR spectrum of neat wool fibers (W00) is reported in Figure 6A. The main relevant signals are due to C=O stretching

(between 1700–1600 cm^{-1}) and N-H bending/C-H bending (between 1560–1500 cm^{-1}) of amide I, typical of α-helix, β-structure and disordered region of keratin secondary structure, and cysteinic disulfide bridges stretching (between 1100–1000 cm^{-1}). The broad signal between 3500–3300 cm^{-1} is due to the N-H stretching, whereas the signal at about 3100 cm^{-1} is due to the Fermi resonance between the first overtone of amide II and the N-H stretching. Finally, signals between 2950–2850 cm^{-1} are attributable to the C-CH_3 and C-CH_2 symmetric and asymmetric stretching mode.[44]
The FTIR/ATR spectrum of neat cotton fibers (C00) is reported in Figure 6B. The main relevant signals are due to intermolecular H-bonding stretching of O-H and C-OH functional groups (at ca. 3330 cm^{-1}), aliphatic C-H stretching (at 2906 cm^{-1}), C-O stretching (at ca. 1160 cm^{-1}), and a broad absorption at ca. 1030 cm^{-1} due to the skeletal C-O-C stretching, typical of polysaccharides structures.[12] The spectra of both natural substrates after AgNPs functionalizion are similar to the reference ones, therefore it is possible to assume that 1) the AgNPs loading did not cause any changes in the fibers macromolecular structure, thus confirming the TGA data previously discussed, and 2) AgNPs are bound to the supports via the electrostatic interactions occurring between the BBS functional groups and the natural fibers functionalities.
All the functionalized fibers were analyzed by UV/Vis spectroscopy in the diffuse-reflection mode without any preliminary preparation (Figure 7). The collected spectra were converted from reflectance to absorbance by using the Kubelka–Munk function. Only a slight modification of the spectra background reflected the change of the color from yellow to beige to brown observed for hot and cold deposition of citrate-stabilized AgNPs and for the deposition of BBS-stabilized AgNPs, respectively, whereas the signals observed were almost similar for all the samples. For this reason, only the spectra relative to the hot deposition of BBS-stabilized AgNPs were discussed for the sake of brevity. The presence of AgNPs is witnessed by the typical signal at about 400 nm. The modification of the background profile could be due to some partial agglomeration of AgNPs during the cold deposition which causes the formation of a darker color together with the classical yellow typical of nanosized particles presence. In the case of BBS-assisted synthesis, the brown color is surely due to the presence of BBS.
Quantification of the Ag loading was achieved by means of ICP-AES measurements after digestion of the fibers. The relevant results are reported in Table 1 as mg Ag per g fiber. The amount of AgNPs loaded on both natural substrates corresponds in all cases to about 0.10–0.15 mgg^{-1}. The fluctuation of the data could reflect some inhomogeneity of the loading since only 20 mg of fibers were used for each test, nevertheless BBS seem to allow a higher loading of AgNPs on fibers with respect to citrate when the cold deposition method is applied.

2.4. Ag Release in water: Static versus dynamic conditions

AgNPs release in Milli-Q water from functionalized fibers was preliminarly evaluated by ICP-AES under three different conditions, as described in the Experimental Section: statically, by soaking for 7 and 15 days to simulate the contact of fibers with sweat, and dynamically, by centrifugation to simulate a washing machine treatment. All the fibers, before and after the release procedure, were chemically digested to evaluate both the initial loaded amount of AgNPs and the AgNPs residue after the release tests. External calibration curve is reported in Figure S6. Experimental results are reported in Table 1.
The amount of Ag (mg) was measured with respect to the amount of fibers subjected to release tests (grams). Moreover, the concentration of Ag residues on fibers after release tests was related to the AgNPs initially loaded on fibers and reported in wt. %.
In general, the results of the release tests were affected by some uncertainty because it was not possible to achieve, in 7 or 15 days, an equilibrium between the two phenomena of AgNPs release from the fibers and AgNPs re-adsorption on the fibers. For this reason, one trial carried out after 15 days of soaking evidences a lower release than after seven days (CBC), whereas the other trials did not evidence an univocal trend. Moreover, the small amount of fibers employed for these preliminary tests (20 grams, about 5 cm of fibers) can affect the reproducibility of the

measurements if the deposition was not completely homogeneous. Nevertheless, some general trends can be inferred: 1) Ag is not irreversibly bonded to the fibers but it is partially released after soaking and/or centrifugation; 2) after 15 days of soaking AgNPs are still present on the supports indicating that fibers can be used for at least 360 h maintaining part of the AgNPs; 3) the centrifugation always removes AgNPs from the supports; 4) BBS seem to stabilize better than citrate the AgNPs on the fibers, limiting their release, in particular when the hot deposition method is applied.

3. Conclusions

BBS (biopolymers derived from composted biowaste) have proven to be effective in the reduction of Ag^I for the synthesis of AgNPs of spherical shape. The average size is lower and the size distribution is narrower than AgNPs synthesized by the classical citrate-based Turkevich procedure. The BBS-assisted synthesis produced AgNPs almost stable over time with respect to citrate-stabilized ones.

The methods of deposition upon fabrics applied gave good results and did not damage the fibers, as well as even the hot deposition method carried out at 90°C, as witnessed by TGA and FTIR results. The best results in terms of AgNPs deposition were reached using wool as support, probably thanks to the more stable and specific interactions between Ag and wool cysteine S-H groups. Nevertheless, the hot deposition procedure allowed obtaining nanoparticles with limited aggregation, as suggested by the yellow color of the fibers after deposition, probably because the AgNPs form directly on the fibers where the reactant Ag^+ is trapped and this limits the growth of the synthesized particles.

All the AgNPs-functionalized fibers partially release Ag in water, both under static and dynamic conditions, in amounts enough to impair antibacterial properties. Nevertheless, in vitro tests will be necessary to assess such behavior.

In summary, a method has been proposed to prepare AgNPs-functionalized textiles by using BBS as stabilizers. The method is environmentally friendly and besides allows valorization of a biowaste. The AgNPs are smaller, more uniformly sized and more stable in suspension than particles produced by the Turkevich method, confirming the important role of BBS as a tool for obtaining chemicals.

4. Experimental section

4.1 Materials

Undyed sheep wool fibers (Lana Borghesia) and cotton fibers (Marca Campanula) were selected as substrates. The bio-based substances (BBS-GC) were isolated from urban biowastes sampled from the process lines of ACEA Pinerolese Industriale S.p.A. waste treatment plant in Pinerolo (Italy). The urban biowaste was obtained in the compost production section, from urban public park trimming and home gardening residues aged for more than 180 days (green compost, GC). The process is an advanced system that comprises specific technological facilities, developed by ACEA Pinerolese Industriale S.p.A., and under European validation.[45] Isolation of BBS-GC was performed following a previously reported procedure by treating 50 g of GC with 1 L of 6m NaOH water solution under stirring at 60°C for 4 h.[36] The reaction mix was then separated by centrifugation. The separated liquid phase was concentrated and different fractions were separated through a lab-scale ultrafiltration unit equipped with a membrane (molar mass cut-off 5 kDa). The retentate fraction was then dried at 60°C for 24 h. The BBS-GC thus obtained was about 20–30% of the starting compost. Before using, BBS-GC pellets were grinded. Chemical compositions of such BBS-GC are reported in Table S1. Other reagents used were: Trisodium citrate bihydrate ($Na_3C_6H_5O_7{\cdot}2H_2O$, E. Merck, purity 99%, CAS 6132-04-3), Silver nitrate ($AgNO_3$, Carlo Erba, purity 99.8%, CAS 7761-88-8), Nitric acid (HNO_3, Sigma Aldrich, conc. >65%, CAS 7697-37-2), Hydrogen peroxide (H_2O_2, Sigma Aldrich, conc. >30%, CAS 7722-84-1), and standard silver

solutions prepared from high-purity metallic silver (Ag in 2% HNO_3, Fluka, conc. 1000 mgL^{-1}). All chemicals were used without purification.

4.2 Preparation of AgNPs colloidal suspensions

The AgNPs were prepared following a modified procedure reported in the literature.[10, 24, 46, 47] Briefly, $AgNO_3$ (1 mm aqueous solution, 0.5 L) was heated up to 90°C under mechanical stirring, 10 mL of a 0.1 mgL¢1 aqueous solution containing the reducing agent/stabilizer (pre-heated at 90°C) was added drop-wise to the silver-containing solution. After 12–15 min, the solution turns yellow (thus indicating the formation of AgNPs). After 20 min of reaction time, the AgNPs solution has been cooled down to RT by running water continuing stirring for 1 h. Two different reducing agents/stabilizers were selected: trisodium citrate (Turkevich reference) and BBS. Moreover, four different reaction mixture were investigated, partially substituting the amount of trisodium citrate (expressed in wt.%) with BBS: 1) 0% BBS (100% citrate and 0% BBS); 2) 50% BBS (i.e. 50% citrate and 50% BBS); 3) 75% BBS (i.e. 25% citrate and 75% BBS); 4) 100% BBS (0% citrate and 100% BBS). AgNPs suspensions were coded with the acronym AgNPs X, where X stands for the wt.% of BBS used (namely 0, 50, 75 and 100). AgNPs_0 (i.e. 100% citrate) was taken as Turkevich reference.

4.3 AgNPs loading at the natural fibers surface

Two different AgNPs suspensions were deposited onto the two natural fibers: AgNPs_0 (Turkevich reference) and AgNPs_100. The natural fibers investigated as textile substrates were: sheep wool (W) and cotton (C), an animal protein and a vegetal polysaccharide, respectively. AgNPs loading was performed by dip-coating deposition by means of two different methods, namely, cold and hot deposition. Cold deposition was performed by immersing the natural fibers directly into a previously prepared AgNPs aqueous suspension (cooled at RT) mechanically stirred for 2 h. Afterwards, the functionalized fibers were recovered and air-dried at RT. Hot deposition was performed by immersing the natural fibers into a $AgNO_3$ aqueous solution under mechanical stirring. After 10 min, the solution was heated up to 90°C and then reducing agents/stabilizers were added drop-wise, following the standard procedure. The functionalized fibers were recovered and air-dried at RT. The natural fibers were coded with the acronym XYZ, where X stands for the type of fiber (W for wool and C for cotton), Y represents the AgNPs suspension investigated (C for citrate, B for BBS and 0 for the neat fibers not functionalized), and Z stands for the loading method adopted (C for cold deposition, H for hot deposition and 0 for the neat fibers not functionalized).

4.4 Characterization Methods

High-resolution transmission electron microscopy (HRTEM) was used to evaluate the AgNPs morphology. Micrographs were obtained using a JEOL JEM 3010 instrument (300 kV) equipped with a LaB_6 filament coupled with energy-dispersive X-ray spectroscopy (EDS). For the specimen preparation, a few drops of AgNPs colloidal suspensions were poured on holed carbon-coated copper grids and left to dry overnight. The AgNPs sizes and distributions were evaluated using the software Particule2 (version 2.0) on at least 100 particles.

UV/Vis spectra of the AgNPs colloidal suspensions were recorded using a UV/Vis Varian Cary 300 Scans instrument with a scan interval of 1 nm. The UV/Vis spectra of the AgNPs-functionalized fibers were recorded in the diffuse-reflection mode (integrating sphere and $BaSO_4$ paint as the reference) using a UV–Vis–NIR Varian Cary 5000 instrument in the range 200–700 nm and with a scan interval of 1 nm.

Fourier-transform infrared spectra (FTIR) were recorded in the attenuated total reflection mode (ATR) using a diamond cell for single reflection in a Bruker Vector IFS28 spectrophotometer equipped with a Globar source, a DTGS detector, and working with 128 scans at 4 cm^{-1} of resolution in the range 4000–400 cm^{-1}. ATR-FTIR spectra were obtained on single fibers repeating the acquisition three times.

Thermo-gravimetric analysis (TGA) was carried out using a TA Q600 (TA Instruments). Thermal analyses were performed on fiber sections with a heating ramp of 10 °Cmin^{-1} from RT to 800°C under nitrogen atmosphere. Two replicas were performed for each treatment condition.

4.5 Ag quantification

A preliminary evaluation of the amount of Ag loaded/released was performed using a PerkinElmer Optima 7000 DV model inductively coupled plasma atomic emission spectrometer (ICP-AES), equipped with an Echelle monochromator, a cyclonic spray chamber, and a PTFE Mira Mist nebulizer. The instrumental conditions used were: plasma power 1.3 kW; torch temperature between 7500 and 8000 K; Ar sampling flow 15 Lmin^{-1}. The Ag emission line considered was 328.068 nm. Ag loading was quantified after fibers digestion following the procedure reported by Klemencic et al.[16] Samples were treated by using a mixture of 65% nitric acid (3 mL) and30% hydrogen peroxide (2 mL) in a closed vessel and microwave heated up by means of a High Performance Microwave Digestion Unit mls 1200 Mega MILESTONE with the following thermal program: 5 min at 250 W, 5 min at 400 W, 5 min at 600 W, and 5 min at 250 W. Resulting solutions were filtered and diluted to 20 mL with Milli-Q (Millipore) ultrapure water (18.2 MWcm^{-1}). Release experiments were carried out treating 20 mg of fibers under three different conditions: 1) after soaking in 10 mL of Milli-Q water for 7 days, and 2) 15 days (to simulate the contact with sweat), and 3) after centrifugation (dynamic condition) in 10 mL of Milli-Q water for 20 min at 2000 rpm, to simulate a washing machine treatment. Afterwards, the fibers were recovered, air-dried, and chemically digested to obtain an Ag residue after release. External calibration was performed and the HNO_3/H_2O_2 digestion solution was used as reference background (Figure S6). The quantification of Ag released in water was done using release baths acidified by HNO_3 addition.

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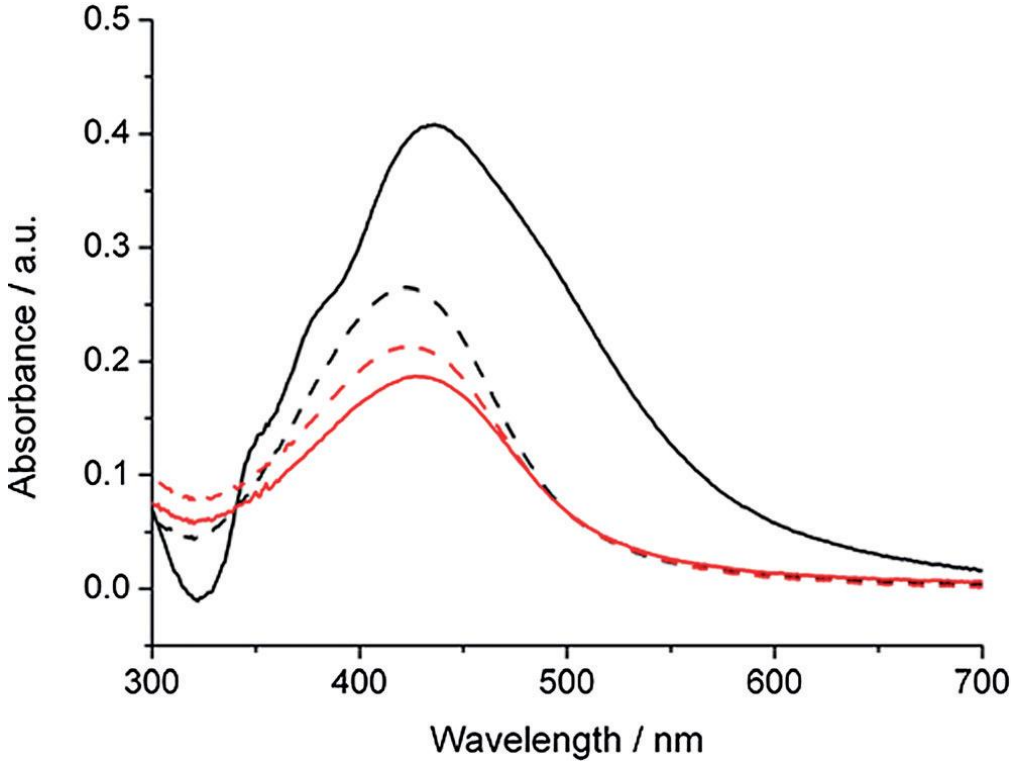

Figure 1. Absorbance UV/Vis spectra in the 300–700 nm range relative to AgNPs colloidal suspensions: AgNPs_0 (black solid line), AgNPs_50 (black dashed line), AgNPs_75 (red dashed line), and AgNPs 100 (red solid line). The AgNPs_50, AgNPs_75 and AgNPs_100 spectra were collected after 1:10 dilution.

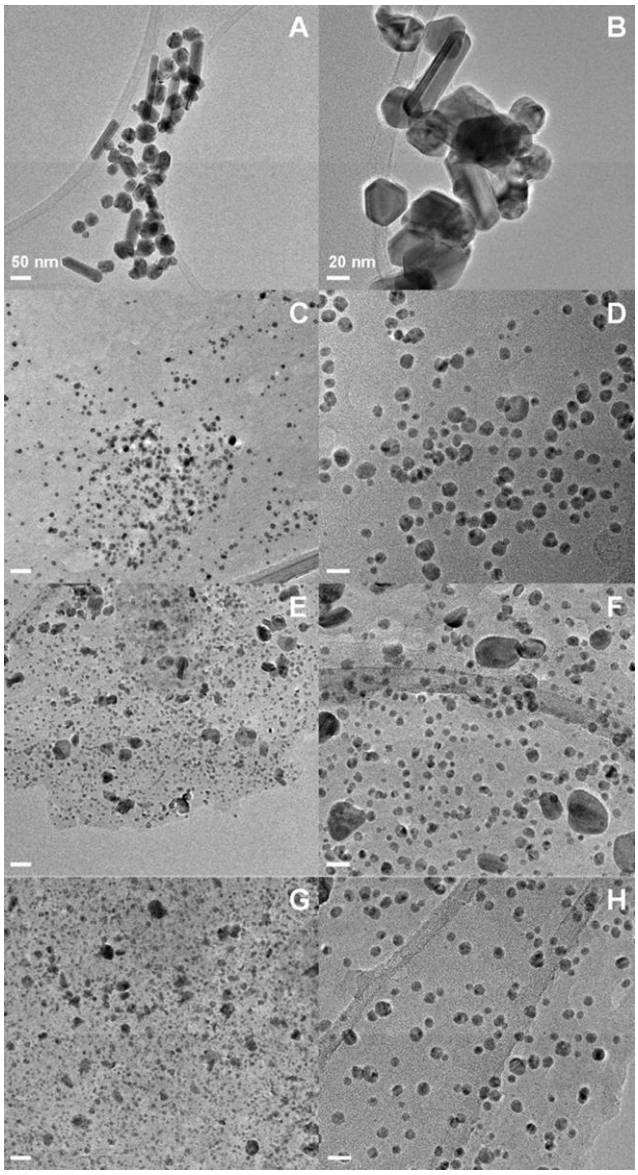

Figure 2. TEM micrographs of AgNPs_0 (A, B), AgNPs_50 (C, D), AgNPs_75 (E, F), and AgNPs_100 (G, H) at low (left) and high (right) magnifications.

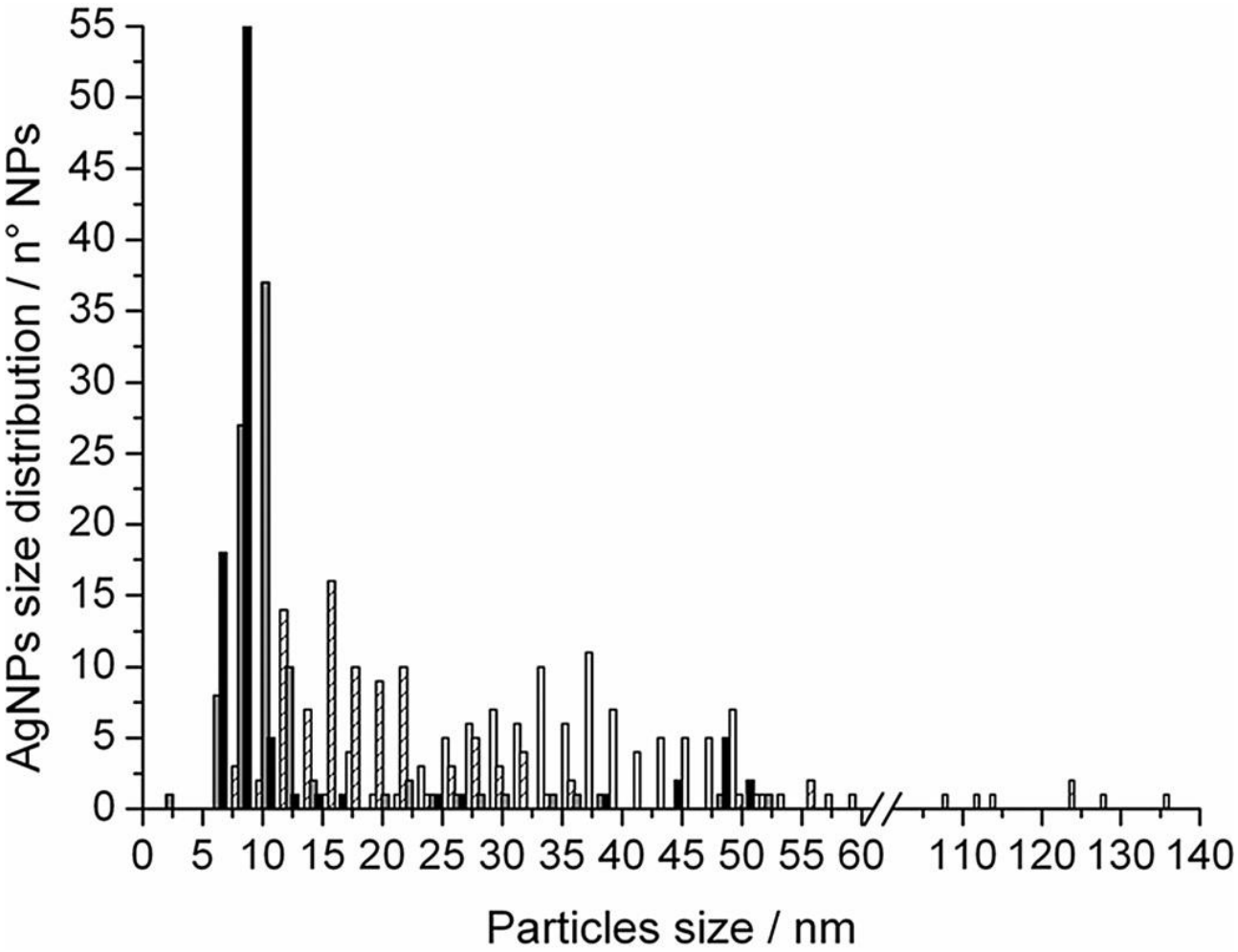

Figure 3. AgNPs size distribution. Legend: AgNPs_0 (white), AgNPs_50 (striped), AgNPs_75 (grey), and AgNPs_100 (black).

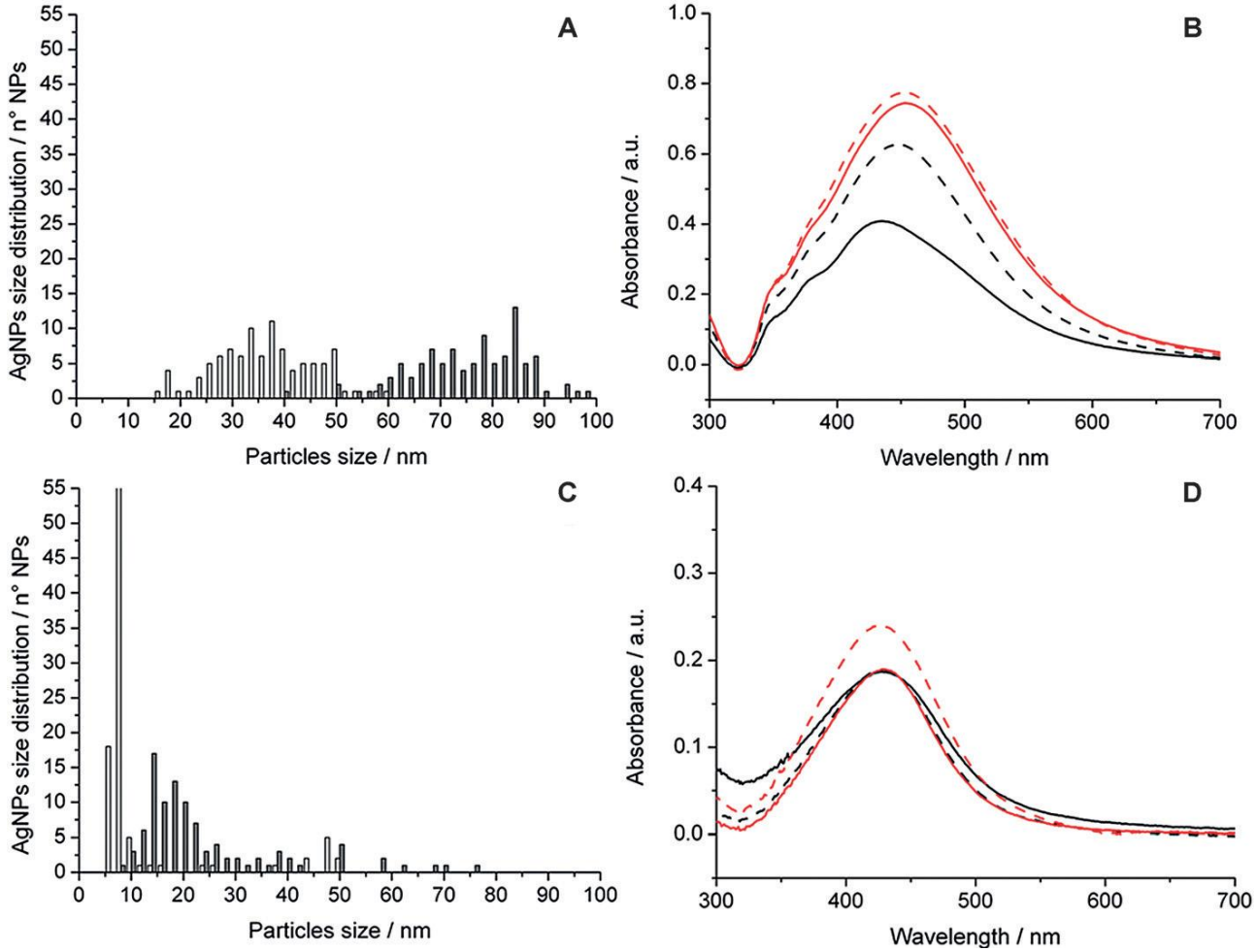

Figure 4. Aging effects on AgNPs suspensions stabilized by citrate (AgNPs_0, top) and BBS (AgNPs_100, bottom). AgNPs size distribution (left) after one day (white histograms) and six months (grey histograms) of aging, and absorbance UV/Vis spectra in the 300–700 nm range (right) after two days (black solid lines), 15 days (black dashed lines), 30 days (red solid lines), and 50 days (red dashed lines) of aging. The AgNPs_100 spectra were collected after 1:10 dilution.

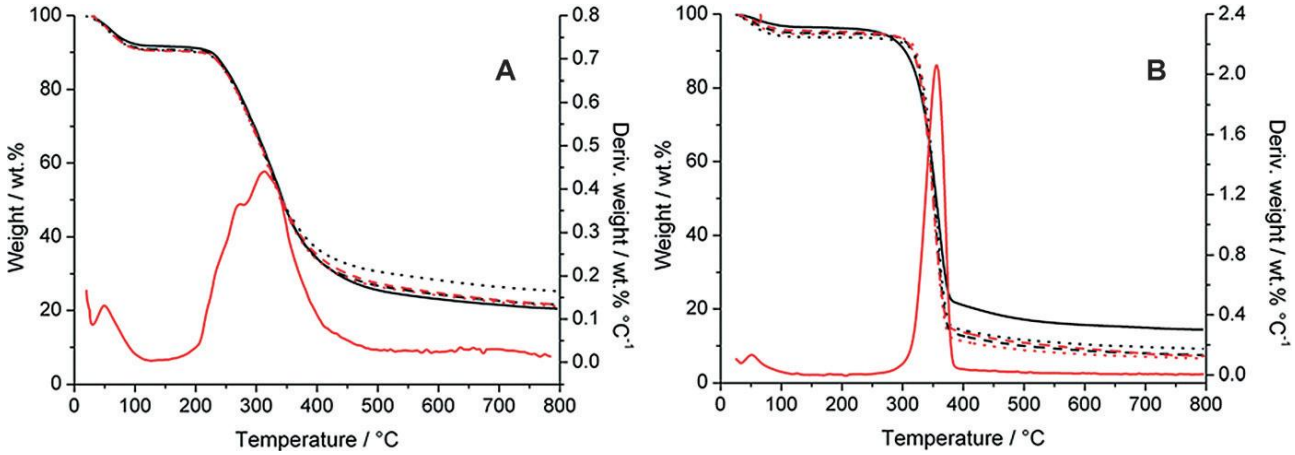

Figure 5. Thermal degradation relative to wool (A) and cotton (B) fibers from RT to 800°C. Comparison between non-treated fibers (black solid line), citrate-stabilized AgNPs-functionalized fibers by cold (black dashed lines) and hot (black dotted lines) deposition, and BBS-stabilized AgNPs-functionalized fibers by cold (red dashed lines) and hot (red dotted lines) deposition. The differential thermal curve of neat natural fibers (i.e. W00 and C00) is also reported (red solid lines).

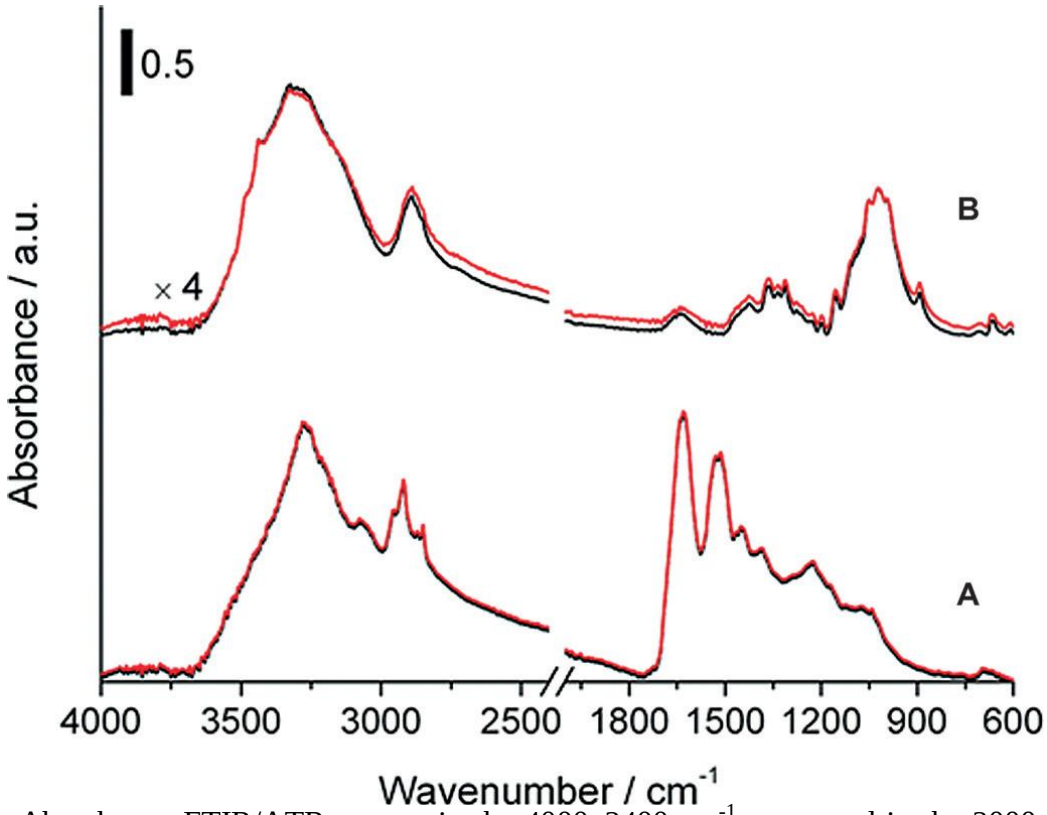

Figure 6. Absorbance FTIR/ATR spectra in the 4000–2400 cm^{-1} range and in the 2000–600 cm^{-1} range relative to wool (A) and cotton (B) fibers. Comparison between non-treated fibers (black solid line) and BBS-stabilized AgNPsfunctionalized fibers by hot deposition (red solid line).

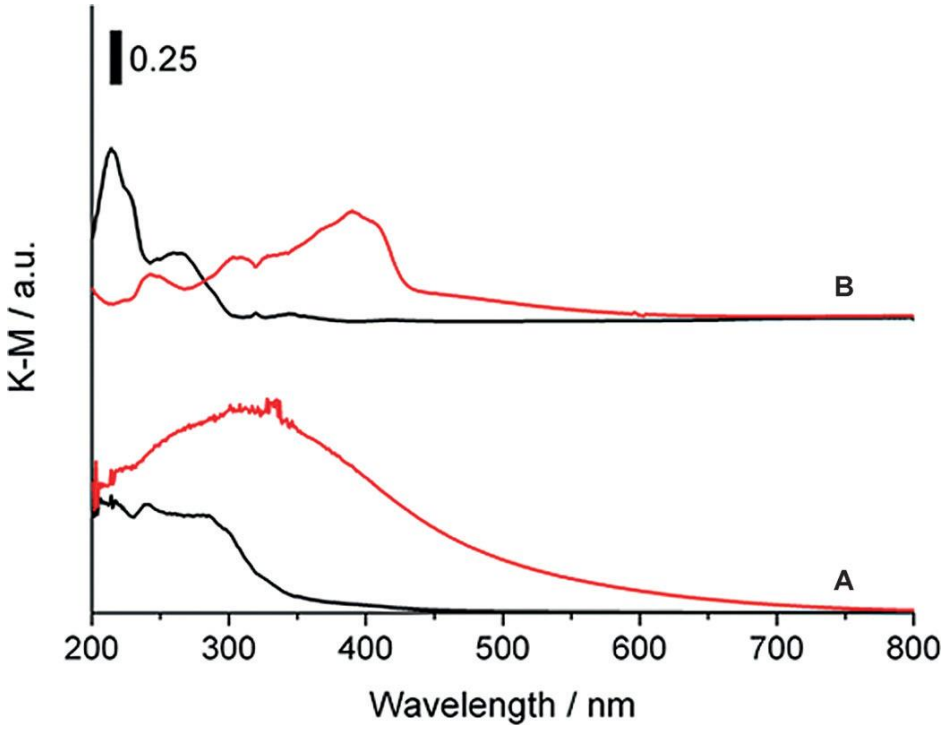

Figure 7. Diffuse-reflection UV/Vis spectra in the 200–800 nm range relative to wool (A) and cotton (B) fibers. Comparison between non-treated fibers (black solid line) and BBS-stabilized AgNPs-functionalized fibers by hot deposition (red solid line).

Table 1. AgNPs residues on fibers after soaking and centrifugation.

Samples	Ag amount loaded on the fibers [mgg^{-1}]	% Ag residue after 7 days of soaking [wt.%]	% Ag residue after 15 days of soaking [wt.%]	% Ag residue after centrifugation [wt.%]
WCC	0.1214	75	30	35
WCH	0.1340	33	35	32
WBC	0.1565	80	28	32
WBH	0.1012	80	65	77
CCC	0.1167	70	23	39
CCH	0.1553	48	25	37
CBC	0.1602	31	55	35
CBH	0.1102	71	56	46