ANTIBACTERIAL ACTION OF SILVER APPLIED ON CELLULOSE FIBERS GRAFTED WITH MONOCHLOROTRIAZINYL-β-CYCLODEXTRIN

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This study aims to compare the antibacterial action of silver in ionic form (Ag^+) with that of silver in a reduced state (Ag^0), when it is in the form of nanoparticles (AgNPs). Silver, in both states, has been studied in terms of its interaction with β-cyclodextrin grafted onto cellulose fibers through the monochlorotriazinyl function. We attempted a correlation of the stability of the complex formed by the two forms of silver with their antibacterial action and resistance to washing of the silver attached to fiber.

Keywords: silver, monochlorotriazinyl-β-cyclodextrin, dextrose, UV spectra, Benesi-Hildebrand equation, antibacterial activity

INTRODUCTION

Making changes in textile surfaces by grafting cyclodextrin (CD), whose hydrophobic cavities can host various substances to be released slowly, requires a prior study on the conditions of formation of cyclodextrin inclusion products; in this case, we refer to studying the interaction of silver in oxidation states of Ag^+ and Ag^0 with monochlorotriazinyl-β-cyclodextrin (MCT-β-CD).

Substances hosted by cyclodextrins can play different roles in applications in pharmaceutical, cosmetic or chemical industries, food technology, agriculture and zooculture. Also, cyclodextrins have found various applications in the textile industry, in increasing wear comfort, making medical textiles etc. In order to increase the wearing comfort, cyclodextrins are used to improve the hygroscopicity[1] and antistatic properties, to capture odors and sweat or release for a long time previously encapsulated odors.[2] In the field of medical textiles, cyclodextrins grafted onto woven fabrics, knitted fabrics or non-woven materials can be used for encapsulation of antibacterial substances effective in reducing or eliminating hospital-acquired infections caused mainly by bacteria resistant to antibiotics. Treating with antimicrobial products the textiles used in this field is an opportunity to apply the properties to slowly release the substances included in the CD. Fabrics, especially natural fibers such as cotton, can be favorable environments for pathogenic microorganism growth. In contact with human skin, these materials provide ideal conditions for breeding and growth of bacteria, due to the large surface area, which retains moisture and nutrients from skin secretions.[3]

The treatment of the textile materials used in hospitals with silver in various forms provides an opportunity for fighting the antibiotic-resistant pathogens. It is believed that the affinity of silver for sulfur and phosphorus is the explanation of this effect. Given the sulfur protein abundance in the bacterial cell membrane, silver is able to react with these proteins and affect bacterial growth. In ionic form (Ag+), silver can interact with nucleotide phosphate phosphorus in DNA, resulting in the disruption of its replication and the inhibition of bacterial multiplication. This property encourages the use of silver on bandages used to treat wounds.[3] Also, studies based on proteomics analysis of the manner small-size AgNPs (below 10 nm) work report that nanoparticles destabilize the outer membrane of *Escherichia coli.* It has been noted that the smaller the size of AgNPs, the more pronounced

is the antibacterial activity and the more reduced is the toxic effect on human cells.[4] Given the importance of the size of AgNPs obtained in the chemical reduction of Ag^+, which is considered the most popular method due to its simplicity, one must remember the factors influencing the growth of silver nanoparticles: the nature of the reducing agent, the precursor concentration, the pH value and the nature of dispersant.

When using a strong reducing agent, such as $NaBH_4$ or N_2H_4, fine particles are obtained quickly and if the precursor, such as $AgNO_3$, has high concentration, the dispersant can not act because of its limit of diffusion at the surface of colloidal silver particles, which must be protected against agglomeration. In order to restrict the uncontrolled growth of silver particles, the use of high concentrations of silver ions is avoided, which in exchange results in a low reaction yield. When using a weaker reducing agent, such as glucose, the speed of the reduction reaction decreases and the obtained particles are smaller and more uniform. If one uses dextrose as a reducing agent, the hydroxyl ions play an important role especially in the first phase of reaction, when the yield is proportional to the amount of NaOH added.[5] The influence of hydroxyl ions is shown by the fact that, depending on their concentration, the reduction reaction can follow two paths (Fig. 1). In version A, the hydroxyl ion performs a nucleophilic attack on the aldehyde carbon of dextrose, the gluconate ion being formed, and then the reduction of silver ions occurs. In version B, Ag^+ ions react with the hydroxyl ions, Ag_2O being formed, which is then reduced by glucose. At low pH (for example pH=8), the reaction follows according to version A and, as pH increases (for example pH=12), path B becomes dominant.[6]

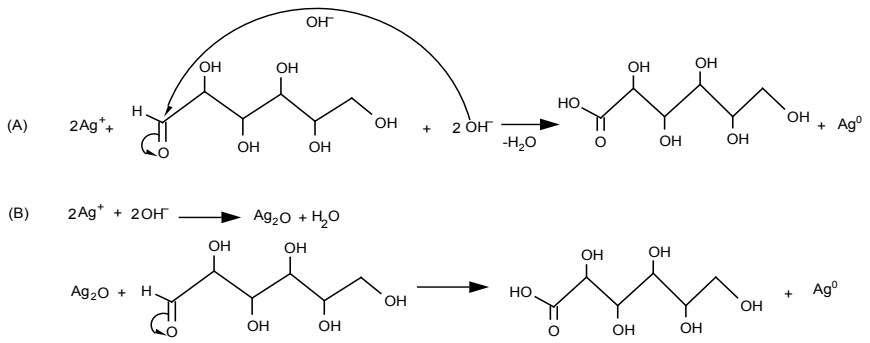


Figure 1: Reduction of Ag^+ with dextrose: A – at low pH; B – at high pH[6]

The dispersing agent has two functions:[6]

- to form a complex with the precursor (Ag^+, in this case) and adjust the speed of the reduction reaction;
- to prevent agglomeration of particles.

One can use dispersants, such as sodium dodecyl sulfate,[7] cetyl-trimethyl ammonium bromide,[4] polyvinyl-pyrrolidone,[5] etc. Given the large number of peripheral hydroxyl groups of cyclodextrins, they can be considered as an ideal agent for AgNPs stabilization.[8] If the reduced form of silver is found as AgNPs, which, given their large size, do not fit into the cyclodextrin cavity and are assumed to be chemically adsorbed in the crown of hydroxyl groups, silver ions (Ag^+) would be able to enter the cyclodextrin cavities.[9]

The objective of this article has been to study the interaction of silver ions with MCT-β-CD grafted onto cotton and to reduce the Ag^+ ions *in situ*, thus avoiding the use of other agents of dispersion (the process is therefore completely green) and obtaining higher resistance of the silver attached to fibers to repeated washings.

The comparative testing of the antibacterial activity of the silver found on the MCT-β-CD grafted and, respectively, ungrafted fabrics, in both oxidation states of silver, and the study of the antibacterial action of the residual silver, in both oxidation states, on both MCT-β-CD grafted and ungrafted fabrics, after a number of standard washings, allowed us to draw conclusions on the correlation of the obtained results with the stability of the silver complexes formed as described above. The assessment of the degree of grafting (Y) was performed by the gravimetric method by weighing the samples before and after

the treatment, after a prior conditioning, using Equation (1):

$$Y = \frac{M_t - M_0}{M_0} \cdot 100, \% \quad (1)$$

where M_t and M_o are the mass of the treated sample and, respectively, the initial mass of the sample.

EXPERIMENTAL

Materials and methods

Preparation of fabrics

Raw fabric of 100% cotton was used, with a specific weight of 130 g/sqm, purchased from IASITEX SA (Romania), alkaline treated for two hours with 20 g/L sodium hydroxide, 5 g/L sodium carbonate, 5 g/L Lavotan DSU (Bezema, Switzerland), 2 g/L trisodium phosphate, liquor ratio 1:20. The alkaline treatment was preceded by desizing by impregnation at 65 °C with 7 g/L Beisol DO conc. (Bezema, Switzerland) and 5 g/L Lavotan DSU, liquor ratio 1:10, followed by storage for 2 hours at 50-60 °C. As final treatments we used repeated washings conducted with hot (90 °C) and cold water until a pH of 6.5-7 was reached. The cleaned fabric was grafted with MCT-β-CD (Wacker Chemie, Germany), following a procedure described in other papers.[10] Silver nitrate (Chimopar, Romania) was used, Ag^+ ion source and dextrose (Fluka, Switzerland) as reductant.

Determination of stability constants (Ks) for complexes Ag^+/MCT-β-CD and Ag^0/MCT-β-CD

To demonstrate the formation of complex Ag^0/MCT-β-CD, the UV-Vis spectral determination of stability constant in aqueous solution has been used. Spectra were obtained using a Single Beam Scanning Spectrophotometer CAMSPEC and quartz cuvettes of 1 cm size. The constant of stability was calculated using the Benesi-Hildebrand equation (2), which establishes a linear relationship between inverse variation of silver absorbance and inverse concentration of MCT-*β*-CD, a relationship of the form y = *a*x + *b*, where, for Ag^0, $b=1/\Delta\varepsilon\cdot[Ag^0]$ determined as intercept of the y axis, $a = 1/\Delta\varepsilon\cdot[MCT\text{-}CD]\cdot[Ag^0]$ determined as the slope of the line obtained.

$$\frac{1}{\Delta A} = \frac{1}{\Delta\varepsilon\cdot[MCT-CD]\cdot[Ag^0]\cdot K_{sAg^0}} + \frac{1}{\Delta\varepsilon\cdot[Ag^0]} \quad (2)$$

where Δε is a constant representing the difference between molar absorptivity of Ag^+ in the absence and presence of MCT-β-CD, respectively, $[Ag^0]$ is the molar concentration of Ag^0, and [MCT-CD] is the molar concentration of MCT-β-CD; it is noted that $[Ag^0]$ is also a constant and so the equation has the form y = *a*x + *b*, where *b* is expressed as $1/\Delta\varepsilon\cdot[Ag^0]$ and determined as the intercept of the y axis, and $a = 1/\Delta\varepsilon\cdot[MCT\text{-}CD]\cdot[Ag^0]$ is the slope of the line obtained. From the ratio of the expressions for *a* and *b*, it follows that $K_{sAg^+}=b/a$.

To determine the stability constant of the complex formed by Ag^+ with MCT-β-CD using a direct method of potentiometric titration of silver ions with an aqueous solution of MCT-β-CD.[11]

The formation of a complex of a ligand (L) and a cation, in this case Ag^+, can be written as:

$$Ag^+ + L \rightleftharpoons Ag^+L \quad (3)$$

where Ag^+L is the complex formed.

The stability constant of the complex formed (K_{sAg^+}) is defined as:

$$K_{sAg^+} = \frac{[Ag^+L]}{[Ag^+]\cdot[L]} \quad (4)$$

Applying the conservation mass law for silver ions and ligand, one can obtain:

$$c_{Ag^+} = [Ag^+] + [Ag^+L] \quad (5)$$

$$c_L = [L] + [Ag^+L] \quad (6)$$

where c_{Ag+} and c_L are the initial concentrations of silver ions and ligand.

From (5) and (6), it follows that:

$$[Ag^+L] = c_{Ag^+} - [Ag^+] \quad (7)$$

$$[L] = c_L - [Ag^+L] \quad (8)$$

Substituting the expressions for $[Ag^+L]$ and [L] in (4), we obtain:

$$K_{sAg^+} = \frac{c_{Ag^+} - [Ag^+]}{[Ag^+]\cdot(c_L - c_{Ag^+} + [Ag^+])} \quad (9)$$

Applying the Nernst equation, we obtain:

$$[Ag^+] = c_{Ag^+}\cdot 10^{\Delta U/D} \quad (10)$$

where ΔU is the potential difference recorded during titration and D is a constant defined by the expression:

$$D = -2{,}303\cdot RT/nF \quad (11)$$

where R is the universal constant of ideal gas, T is the absolute temperature, n is the electric charge of cations and F is Faraday's constant. For silver electrode at 25 °C, the value of D is 59.16 mV.

Substituting expression (10) in (9) and taking into account the dilution of $AgNO_3$ solution during titration by adding a solution of ligand volume V, we obtain:

$$K_{sAg^+} = \frac{\frac{c_{Ag^+}\cdot V_{Ag^+}}{V_{Ag^+}+V} - c_{Ag^+}\cdot 10^{\frac{U-U_0}{D}}}{c_{Ag^+}\cdot 10^{\frac{U-U_0}{D}}\left(\frac{c_L\cdot V}{V_{Ag^+}+V} - \frac{c_{Ag^+}\cdot V_{Ag^+}}{V_{Ag^+}+V} + c_{Ag^+}\cdot 10^{\frac{U-U_0}{D}}\right)} \quad (12)$$

To calculate the stability constant for the formation of a 1:1 complex, one must consider the potential only corresponding to an excess of ligand relative to Ag^+. At other ratios of ligand/Ag+ during titration, other complexes may occur to a small extent, consisting of two Ag^+ ions for a ligand molecule or other stoichiometric variants.[11]

Reduction of Ag^+ ions directly on the fabric

Two equal samples of fabric, weighing 1 g, one ungrafted (sample 1) and another grafted with MCT-β-CD (sample 2) are introduced, separately, into two vials of 20 ml and impregnated with 6 ml of 0.01 M silver nitrate solution and then stored in the dark at 50 °C for one hour; and then are treated with 6 ml of 0.01 M dextrose at 50 °C for one hour. Then 6 ml of 0.1 M sodium hydroxide is added and the samples are stored for another hour at 50 °C. The two samples are washed separately for 30 minutes in 100 ml distilled water at a temperature of 70 °C. The fabric samples treated with silver are divided into two halves. One half of each is tested in terms of antibacterial activity, compared to the fabric ungrafted with MCT-β-CD and untreated with silver. The other half of the samples treated with silver are subjected to 10 repeated washings (soaping) – ISO 105-C10:2006, test C (60 °C, 30 min) –, and tested under the same conditions, in terms of antibacterial activity (samples 1' and 2').

Treatment of fabric with silver nitrate

Two equal samples of fabric, weighing 1 g, one grafted with MCT-β-CD (sample 4) and one ungrafted (sample 3) are introduced, separately, into two vials of 20 ml volume and impregnated with 6 ml of 0.01 M silver nitrate solution and 12 ml distilled water, then stored at room temperature for 24 hours in the dark. The two samples are washed separately for 30 min in 100 ml distilled water at a temperature of 70 °C. Then the procedure described above is followed.

Characterization by infrared spectra of silver treated fabric

We tried to highlight the presence of silver in the two forms of oxidation, on the MCT-β-CD grafted and, respectively, on ungrafted cotton fabric, using the infrared spectroscopy technique accompanied by attenuated total reflection (FTIR-ATR), suitable for textile surfaces. FTIR-ATR spectroscopic technique allows the analysis of solids surface using a diamond tipped device, "Golden Gate" Diamond ATR-IR (SPECAC), attached to Bruker Vertex 70 spectrophotometer.

Microbiological investigations

The microbiological investigations consisted in testing the antibacterial activity of the samples, represented by cotton fabrics treated with Ag^0 and Ag^+, respectively. The test organisms were Gram-positive coccus (represented by *Staphylococcus aureus* ATCC 25923) and Gram-negative bacillus (*Escherichia coli* ATCC 25922) from the collection of the Microbiology Laboratory (Faculty of Biology, "Alexandru Ioan Cuza" University of Iasi, Romania). In order to achieve this purpose, the Kirby-Bauer diffusimetrical method[12] was used. The 18-hour bacterial cultures were obtained from bacterial inoculum, which was standardized according to McFarland scale, yielding 10^7-10^8 CFU/ml. The culture medium used (LB) was inoculated with the inoculum; after that, discs (represented by textiles subjected to different treatments) with a diameter of 1 cm were applied on the surface of the medium. The assessment of antibacterial activity was carried out after 24 hours of incubation at 37 °C, by measuring the inhibition zones around the textile disks.

RESULTS AND DISCUSSION

MCT-β-CD grafting on cotton fabric

The degree of grafting calculated with relation (1) was of 5% functionalised cyclodextrin reported to fabric.

Ks_{Ag0} determination

We prepared a number of six solutions of reduced silver as described in *Reduction of Ag^+ ions directly on the fabric*, but without using any fabric. The silver concentration remained constant (0.15 mol/L) and each solution contained increasing amounts of MCT-β-CD ($0.025 \cdot 10^{-3}$-$0.150 \cdot 10^{-3}$ mol/L). Figure 2 shows that the maximum absorbance of Ag^0, at wavelengths of 419-439 nm, clearly decreases with an increasing concentration of cyclodextrin. This phenomenon could be explained by chemical sorption of Ag^0 nanoparticles in the crown of hydroxyl groups of cyclodextrin. The reduced state of silver represents nanoparticles resulting from nucleation and growth process and, because of their large size (the diameter of a single Ag atom is 0.344 nm), they do not fit in the cyclodextrin cavity (β-cyclodextrin cavity has a diameter of max. 0.62 nm). The peaks appeared in the range 419-439 nm are due to the phenomenon named *surface plasmon resonance* of electrons in the conduction band of silver particles. The absorption in the visible domain indicates the formation of AgNPs.[13] Peak intensity is related to the number of AgNPs formed, increasing as a function of it. AgNPs chemical sorption to hydroxyl groups of cyclodextrin may have two opposite effects on the spectrum: on the one hand, an increase in the electron density of silver nanoparticles as a result of electron donation, resulting in peak shifting toward a lower wavelength, on the other hand, the formation of dipoles at the particle surface, leading to peak shifting toward larger wavelengths.[14] One can notice from Figure 2 that in this case the second effect is predominant.

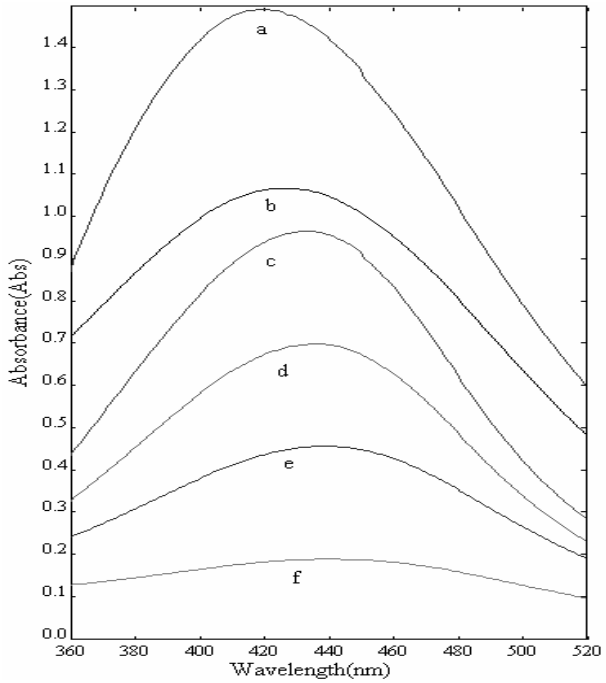


Figure 2: Decrease of absorbance for colloidal Ag^0 (0.15 mol/L) by adding $0.025 \cdot 10^{-3}$, $0.050 \cdot 10^{-3}$, $0.075 \cdot 10^{-3}$, $0.100 \cdot 10^{-3}$, $0.125 \cdot 10^{-3}$, $0.150 \cdot 10^{-3}$ mol/L MCT-β-CD (a→f)

Table 1
Electrochemical potential U for potentiometric titration of $AgNO_3$ solution ($0.6 \cdot 10^{-3}$ mol/L, V = 20 ml) with 0.01 M MCT-β-CD solution, and stability constant Ks for complex Ag^+/MCT-β-CD

V [ml]	U [mV]	$Ks \cdot 10^6$
3.0	200.5	3.194530
3.1	201.5	3.146583
3.2	202.5	3.107956
3.3	203.4	3.065531
3.4	204.6	3.066151
3.5	205.3	3.013877
3.6	206.3	3.002966
3.7	206.8	2.939520
3.8	207.4	2.893265
3.9	208.0	2.851953
4.0	208.5	2.804155

Another possible explanation for the decrease of Ag^0 absorbance by increasing amount of MCT-β-CD added could be silver reduction just in the cyclodextrin cavity with retaining an increased number of Ag^0 atoms in these cavities, proportional to the amount of extra cyclodextrin added. The Ag^0 atoms leaving the cavity are subjected to the rule of nanoparticle nucleation and growth, the generated peaks becoming lower as their number decreases.

The stability constant of the reduced silver inclusion product was calculated using the spectra from Fig. 2 and the Benesi-Hildebrand equation (2), which give $Ks_{Ag0} = 361$ mol^{-1} (Fig. 3).

Determination of Ks_{Ag+}

Potentiometric titration allows the direct determination of activity for the cations remaining in solution (non-complexed). Equal volumes (V_{Ag+} = 20 ml) of silver nitrate solution with a

concentration of c_{Ag+} = 0.6•10^{-3} mol/L were introduced in two thermostatic (25 °C) compartments joined by a bridge with electrolyte, where a salt was used, which does not influence complex formation, namely tetraethylammonium perchlorate ($C_8H_{20}N^+ClO_4^-$). In the two compartments, two identical silver electrodes were introduced and connected to the potential recorder (Newport 8040). Titration was done in one of the compartments equipped with magnetic stirrer, using a Dosimat E 535-Metrohm Herisau (Switzerland) device at a speed of 0.1 ml/min.

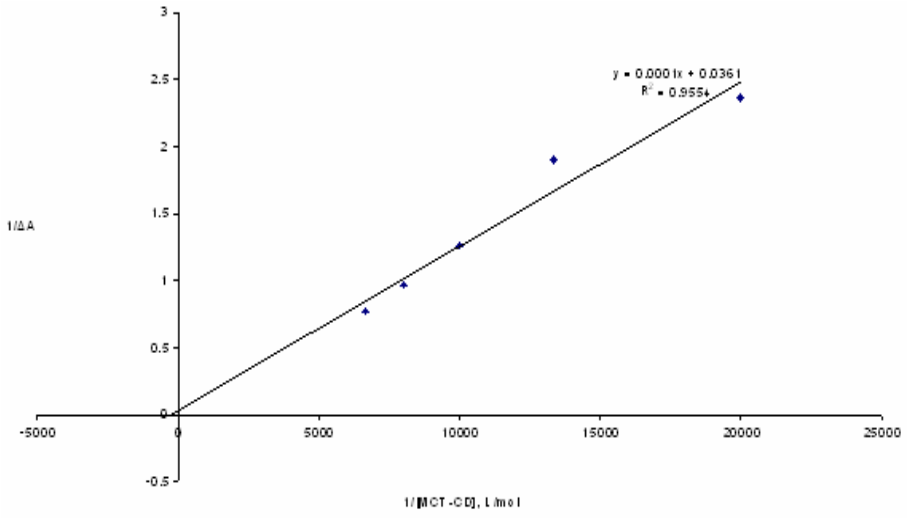


Figure 3: Representation of linear variation of 1/ΔA in terms of 1/[MCT-CD] for Ag^0 inclusion product with MCT-β-CD

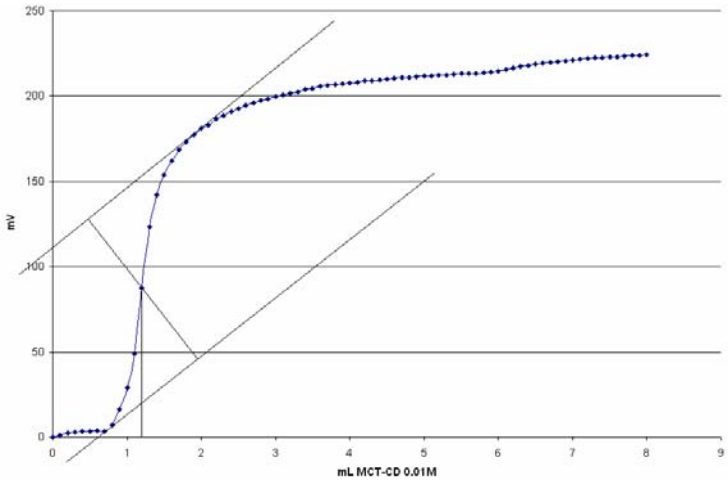


Figure 4: Potentiometric titration curve of 0.6·10^{-3} mol/L $AgNO_3$ solution with 0.01 M MCT-β-CD solution

The equivalence point determined graphically (Fig. 4) at approx. 1.2 ml of 0.01 M MCT-β-CD solution corresponds to the calculated one, that is the amount of Ag^+ contained in the 20 ml of 0.6•10^{-3} mol/L $AgNO_3$ in the titration compartment, indicating the formation of a 1:1 complex. Considering the portion of the titration curve in Fig. 4, between 3 and 4 ml of 0.01 M MCT-β-CD titrated, we obtain the values for K_{sAg+} (Table 1) based on relation (12). The average of the calculated values for logK_{sAg+} is 6.487.

Infrared spectra

When comparing the FTIR-ATR spectra (Fig. 5) of the raw material untreated with silver (a),

the fabric grafted with MCT-β-CD and treated with Ag^+ (b) and the fabric grafted with MCT-β-CD and treated with Ag^0 (c), performed within the absorption range of the hydroxyl groups (stretching vibration) of cyclodextrin (3400-3600 nm), it was found that the peak at 3510 nm disappeared in the case of the fabrics treated with Ag^+ (b) and Ag^0 (c). This fact can be interpreted by Ag^+ and AgNPs binding to hydroxyl oxygen of cyclodextrin grafted on cotton.[3,9]

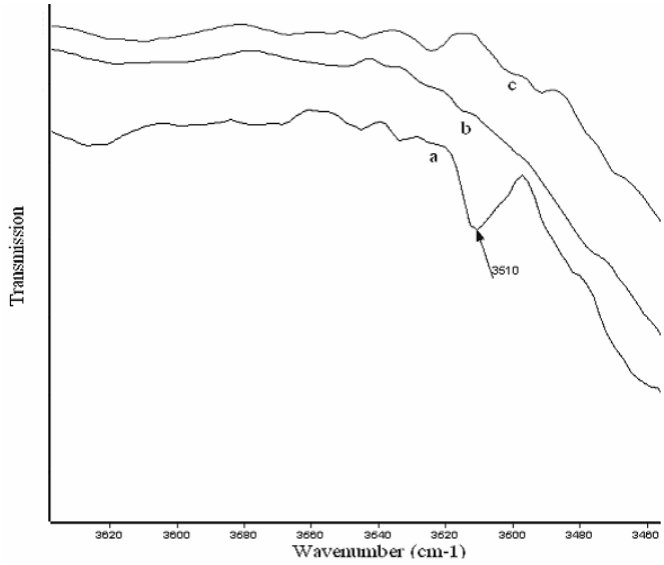


Figure 5: FTIR-ATR spectra of control fabric (a), fabric grafted with MCT-β-CD treated with Ag^+ (b), fabric grafted with MCT-β-CD treated with Ag^0 (c)

Testing of antibacterial activity

After the performed investigations (Figures 6 and 7, Table 2), no antibacterial effect was observed in the case of both fabrics ungrafted with MCD-β-CD and treated with Ag^0 or Ag^+, as well as their counterparts washed 10 times, the diameter of inhibition zones being of 0 mm.

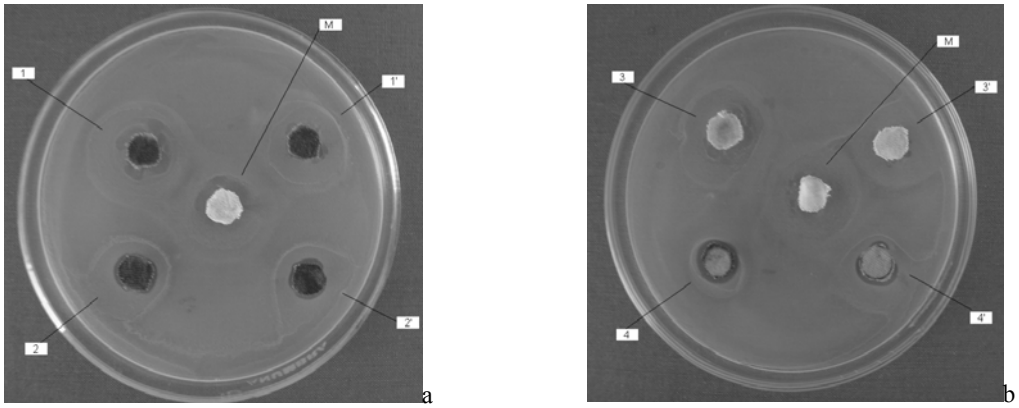
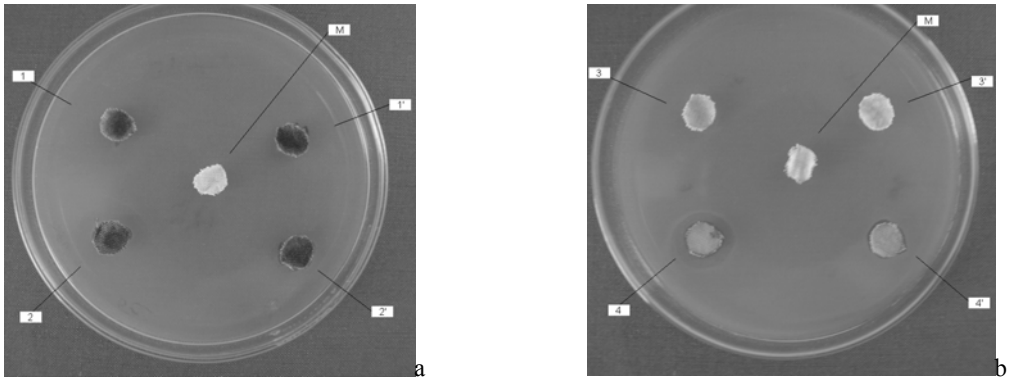


Figure 6: Antibacterial activity of fabrics treated with Ag^0 (a) and Ag^+ (b) against *Staphylococcus aureus* ATTC-25923 (1, 3 – fabrics ungrafted with MCT-β-CD; 1', 3' – fabrics 1, 3 washed 10 times; 2, 4 – fabrics grafted with MCT-β-CD; 2', 4' – fabrics 2, 4 washed 10 times; M – control fabric)



a b

Figure 7: Antibacterial activity of fabrics treated with Ag^0 (a) and Ag^+ (b) against *Escherichia coli* ATTC-25922 (1, 3 – fabrics ungrafted with MCT-β-CD; 1', 3' – fabrics 1, 3 washed 10 times; 2, 4 – fabrics grafted with MCT-β-CD; 2', 4' – fabrics 2, 4 washed 10 times; M – control fabric)

Table 2
Antibacterial activity of cotton fabric treated with silver

No. sample	Type of sample	Inhibition zone diameter (mm)	
		Staphylococcus aureus ATTC-25923	*Escherichia coli* ATTC-25922
1	Ungrafted fabric, treated with reduced silver (Ag^0)	0	0
1'	Fabric 1, washed 10 times	0	0
2	Fabric grafted with MCT-β-CD, treated with reduced silver (Ag^0)	10	8
2'	Fabric 2, washed 10 times	11	9
3	Ungrafted fabric, treated with silver nitrate (Ag^+)	0	0
3'	Fabric 3, washed 10 times	0	0
4	Fabric grafted with MCT-β-CD, treated with silver nitrate (Ag^+)	12	15
4'	Fabric 4, washed 10 times	13	11
M	Control fabric, ungrafted, untreated with silver	0	0

Research results have demonstrated the antibacterial action of the fabrics grafted with MCD-β-CD treated with Ag^0 or Ag^+, but also of those washed 10 times. They showed a differential sensitivity of the two organisms to grafted fabrics, with diameters of inhibition zones that ranged between 8-15 mm in the case of species *Escherichia coli* ATCC 25922, and between 10-13 mm in the case of bacterium *Staphylococcus aureus* ATCC 25923.

In both test microorganisms, it was found that the antibacterial effect was slightly higher in fabrics grafted with MCT-β-CD treated with Ag^+, as compared to those treated with Ag^0.

The preservation of the antibacterial action even after washing the grafted fabric proves a good fixation of silver and cyclodextrin functionalized on cellulose fiber.

In the case of control sample represented by the fabric ungrafted and untreated with silver, the antibacterial effect was not noticed, the diameter of the inhibition zone being 0.

The differential inhibition of growth and the multiplication of the two bacterial species under the action of the test samples could be correlated with the different composition and ultrastructure of the cell wall of the two species, which confers a hydrophilic character to Gram-negative bacteria

(*Escherichia coli*) and a hydrophobic character to Gram-positive bacteria (*Staphylococcus aureus*).

By correlating the stability constants of the complexes formed by the MCT-β-CD with Ag^0 and Ag^+ ($logKs_{Ag0}$ = 2.557 and $logKs_{Ag+}$ = 6.487, respectively) with the results obtained when testing antibacterial activity, one can deduce that the greater stability of complex Ag^+/MCT-β-CD, compared to complex Ag^0/MCT-β-CD, leads to lower removal of Ag^+ than of Ag^0 at the first rinsing after the treatment of fabric with silver in the two states of oxidation. At the same time, after ten washings, the inhibition zones remain close to the baseline, maintaining the advantage for Ag^+. The advantage of silver inclusion in cyclodextrin grafted onto fabric is evident, in comparion with silver application on an ungrafted fabric, regardless of the oxidation state. The fact that for the ungrafted fabrics treated with silver in the two oxidation states no antibacterial effect was noticed could be the result of the removal of most of the silver during the first rinse. There are opinions that the high antibacterial action of AgNPs can be attributed to the cyclodextrin, which, on the one hand, contributes to the formation of small nanoparticles by reducing Ag^0 aggregation and, on the other hand, by its bacterial degradation, causes the release of silver as Ag^+, a phenomenon called the Trojan Horse.[4,7]

CONCLUSION

We managed to get AgNPs *in situ* on cotton fabric, through a completely ecological process, without using dispersants. The role of the dispersant was played by functionalised cyclodextrin grafted on cellulose fiber.

FTIR-ATR spectra of the fabric grafted with MCT-β-CD and treated with Ag+, respectively Ag^0, compared with control fabric ungrafted with MCT-β-CD and untreated with silver, indicate the existence of links between hydroxyl oxygen of grafted cyclodextrin and silver ion, or as nanoparticles respectively.

Fabrics grafted with MCT-β-CD and treated with Ag^0 or Ag^+, respectively, have antibacterial activity, an effect that was observed for both test organisms, *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923.

The antibacterial effect persists for grafted fabrics, washed 10 times, the diameter of the inhibition zones being close to that of the original fabric, not subject to washing.

A correlation was found between the size of stability constant of the complexes formed by MCT-β-CD with silver in the two oxidation states (Ag^0 and Ag^+), the antibacterial activity and the resistance to washing.

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REFERENCES

[1] V. Popescu, E. I. Muresan and A.-M. Grigoriu, *Carbohyd. Polym.*, **86**(**2**), 600 (2011).
[2] A. G. Grechin, H.-J. Buschmann, E. Schollmeyer, *Text. Res. J.*, **77**(**3**), 161 (2007).
[3] R. M. El-Shishtawy, A. M. Asiri, N. A. M. Abdelwahed and M. M. Al-Otaibi, *Cellulose*, **18**, 75 (2011).
[4] J. Garcia-Barrasa, J. M. Lopey-de-Luyuriaga and M. Monge, *Cent. Eur. J. Chem.*, **9**(**1**), 7 (2011).
[5] Y.-C. Lu and K.-S. Chou, *J. Chin. Inst. Chem. Eng.*, **39**, 673 (2008).
[6] H. Wang, X. Qiao, J. Chen and S. Ding, *Colloid. Surfaces A*, **256**, 111 (2005).
[7] S. Jaiswal, B. Duffy, A. K. Jaiswal, N. Stobie and P. McHale, *Int. J. Antimicrob. Agents*, **36**, 280 (2010).
[8] C. George, S. Kuriakose, B. Prakashkumara and T. Mathew, *Supramol. Chem.*, **22**, 511 (2010).
[9] M. Bajpai, P. Gupta and S. K. Bajpai, *Fiber Polym.*, **11**(**1**), 8 (2010).
[10] O. Popescu, R. M. Diaconescu, A. Grigoriu, E. Vasluianu, *Ind. Textila*, **2**, 68 (2012).
[11] H.-J Buschmann., E. Cleve, E. Schollmeyer, *GIT Fachz. Lab.*, **36,** 861 (1992).
[12] G. A. Wistreich, "Microbiology Laboratory", edited by Prentice Hall, Upper Saddle River, New Jersey, 2000.
[13] X. Guangnian, Q. Xueliang, Q. Xiaolin, C. Jianguo, *Rare Metal Mat. Eng.*, **39**(**9**), 1532 (2010).
[14] P. Manikandan, V. Ramakrishnan, *J. Fluores.*, **21**, 693 (2011).