

Application of Nano Silver/Lecithin on Wool through Various Methods: Antibacterial Properties and Cell Toxicity

Hossein Barani¹, Majid Montazer², Nasrin Samadi³, Tayebeh Toliyat⁴,
Mohsen Khorashadi Zadeh⁵, Boudewijn de Smeth⁶

¹Department of Carpet, Faculty of Art, University of Birjand, Birjand, IRAN

²Textile Engineering Department, Amirkabir University of Technology, Tehran, IRAN

³Department of Drug and Food Control, Tehran University of Medical Sciences, Tehran, IRAN

⁴Department of Pharmaceutics, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, IRAN

⁵Department of Medical Biotechnology, Tehran University of Medical Sciences, Tehran, IRAN

⁶Faculty of Geo-Information Science and Earth Observation, University of Twente, Enschede, NETHERLANDS

Correspondence to:

Majid Montazer email: tex5mm@aut.ac.ir

ABSTRACT

Silver nanoparticles (AgNPs) were synthesized with lecithin through a simple chemical reduction method. The prepared AgNPs/lecithin was then loaded into the wool fabric by exhaustion and pad-dry-cure methods. The surface morphology of the loaded wool fabrics was characterized by low-voltage scanning electron microscopy, and the loading efficiency was determined by inductively coupled plasma optical emission spectrometry. Further, the effect of the different lecithin concentrations was examined on the antibacterial activity, cytotoxicity, and color of the loaded wool fabrics. The loaded fabric with AgNPs at a higher lecithin ratio presented higher antibacterial activity due to the higher loading efficiency and smaller nanoparticle size. Also, the morphology of the fibroblast cells in cytotoxicity test was not changed in presence of extracted solution from the treated wool fabrics with different lecithin concentration.

Keywords: Silver Nanoparticle, Lecithin, Antibacterial, Cytotoxicity, Wool.

INTRODUCTION

The design and application of biomaterials in various fields of research have great scientific and practical interests. Biomaterials have unique useful properties such as biodegradability. Combining of nanoparticles with biomaterials engages a special place in synthesis of nanoparticles through chemical reaction. Further,

biomaterials encapsulate nanoparticles due to their multifunctional ligands [1-6].

The ability of surfactants to form defined self-assemble structures has been used as advantages in design and synthesis of AgNPs with various shapes and size [7, 8]. The formed structures by self-assembly surfactant are used as a template of nanoparticles [9]. Lecithin as a surface-active biological lipid forms a liposome membrane structure by exposure to the aqueous phase [10-12]. The polar head groups toward the water phase and hydrophobic hydrocarbon moieties that adhere together in the membrane bilayer structure which can encapsulate nanoparticles [13, 14]. Based on their carrier role, they were being used in several wet processing of textiles such as textile finishing and dyeing. Application of liposome membrane structure in the textile process produces better mechanical properties, washing fastness, and leveling effects [10]. The structure of the cell membrane complex of wool fiber can be changed with liposome absorption due to wool lipid solubilization, which leads to higher accessibility for the dye molecules [15].

The AgNPs was synthesized and entrapped simultaneously in liposome structure in our previous work that reports slow release of silver ions and nanoparticles. The aim of this study was to load entrapped AgNPs within the lecithin into wool fabric

via various methods. Müller et al. reported that lecithin as a biological material reduces the cytotoxicity of poly hexa methylene biguanide hydrochloride. It seems that the lecithin structure reduces the cytotoxicity of the loaded wool fabrics with AgNPs [13].

The morphology and loading efficiency of the treated wool fabrics with AgNPs and different lecithin concentrations were examined by Low-Voltage Scanning Electron Microscopy (LVSEM), and inductively coupled plasma optical emission spectrometry (ICP-OES Liberty). In addition, the effect of the lecithin concentration on the yellowness indexes of the fabrics was studied by monitoring of the reflectance spectra. The antibacterial efficiency of the washed and unwashed fabrics, and their cytotoxicity properties of the treated fabrics was also investigated and the results discussed.

MATERIAL

Fabric Samples

A plain wool fabric composed of 100% wool yarn (48/2 Nm) with a warp and weft density of 17 ends/cm was supplied by Iran Merinous Co, Iran. Firstly, wool fabric was washed with Triton X-100 as a non-ionic detergent (1 g/L) for 30 min at 50 °C (Liquor to goods ratio or L: G = 40:1), then rinsed with tap water and dried at room condition. All the chemical agents were analytical grade. Silver nitrate (AgNO₃ extra pure, >99.8%), sodium borohydride (NaBH₄, 99.9%), and Triton X-100 were purchased from Merck Co. (Germany). Lecithin was received as a gift from Lipoid Co. (Lipoid® S 75; Germany).

METHOD

Preparation of AgNPs Loaded Wool Fabric

The scoured wool fabrics were treated with synthesized AgNPs/lecithin through pad-dry-cure and exhaustion methods.

a) Pad-dry-cure

The scoured wool fabrics were immersed in the AgNPs/lecithin with L:G=40:1 for 15 min. The colloidal AgNPs was prepared according to the previous work [14]. The silver content of the solution was 300 ppm and lecithin to silver concentration ratio was varied including 0, 0.2, 1, and 2. The wool fabrics were then squeezed with pad mangle to obtain 80% wet pick-up and then dried at 80°C for 20 min followed by curing at 130°C.

b) Exhaustion

The solution was prepared with 300 ppm AgNPs/lecithin with various lecithin ratios in L:G=40:1. The scoured wool sample was introduced to the bath at 40°C and the temperature was gradually increased to 90°C for 20 min and remained 30 min.

The treated wool fabrics were washed with distilled water and dried at 80°C for 20 min.

Surface Morphology

The surface morphology of the wool fiber surface and the presence of AgNPs were observed by Low-Voltage Scanning Electron Microscopy (LVSEM, Zeiss Gemini DSM 982). This microscope is the SEM at low energies beam worked at 1 keV. This microscope is a useful tool for nonconductive material which observe surface without gold or carbon coating. The coated layer for increasing conductivity of the loaded fabrics may alter the surface. Therefore, the surface of the loaded wool fabrics was observed without coating by LVSEM.

ATR- FTIR

The chemical changes of wool fiber were analyzed by Attenuated Total Reflection-Fourier Transform Infrared spectrometer (ATR-FTIR, Perkin Elmer Spectrum 100 series). ATR-FTIR spectra were recorded at a resolution of 1cm⁻¹ and the scanning range was 2000–400 cm⁻¹ with an average of 20 scans.

Silver Content in Wool Fabrics

The loading efficiency of AgNPs through different methods and lecithin concentrations was determined on the wool fabric. 0.5 g wool fabric was put 3 h at 600°C. The ash weight was recorded and then dissolved in 1 mL hot concentrated nitric acid for 1 h. The volume was adjusted to 10 mL with distilled water. The Ag content was determined using an inductively coupled plasma optical emission spectrometer (Varian ICP-OES Liberty II) with axial plasma calibrated with five Ag standard solutions over 0.5 to 20 mg/L after a twenty times of extra dilution with water to reach the concentrations within the range of the calibration solutions.

Color Measurement

The reflectance spectra of the samples were recorded in the visible region with Color Eye 7000 A, Gretag-Macbeth. The *K/S* value was calculated according to Eq. (1) [16].

$$\frac{K}{S} = \frac{(1 - R)^2}{2R} \quad (1)$$

where; R, K, and S are reflectance, absorption coefficient and scattering coefficient of the sample, respectively.

The presences of AgNPs on the wool fabric change the color of the loaded fabric to brownish yellow. This color changes can be described by the

yellowness index, which was calculated from spectrophotometer data. The yellowness index of the loaded fabric was determined according to ASTM E313 Eq. (2) under illuminant D₆₅ and 10° standard observer:

$$YI = 100(1.3013X - 1.1498Z)/Y \quad (2)$$

Washing Fastness

The wash fastness of the loaded wool fabrics with AgNPs/lecithin was obtained by washing the fabrics in 1 g/L Triton X100, L:G=40:1 at 50°C for 45 min.

Antibacterial Properties

The antibacterial efficiency of the treated wool fabrics against *Staphylococcus aureus* (ATCC 6538) as a Gram-positive and *Escherichia coli* (ATCC 8739) as Gram-negative bacteria were carried out by two different methods of qualitative and quantitative.

a) Qualitative test

The qualitative test is known as an agar diffusion method based on inhibition zone surrounding the sample, where bacteria growth is inhibited on the inoculated agar plate. This test was carried out according to a modified version of AATCC test method 147-2004.

The surface of petri dishes containing 25 mL Mueller-Hinton agar was seeded individually with bacterial suspensions (10⁸ CFU/mL) by a sterile cotton swab. The specimens 2.5×5.0 cm² was pressed on the inoculated agar plate gently. The prepared petri dishes were incubated at 35°C for 20 h, and diameter of inhibition zone was determined.

b) Quantitative test

The quantitative test is based on determination of viable bacteria which can estimate the antibacterial efficiency of the treated samples in term of bactericidal and bacteriostatic effects. In addition, it is possible to find out the antibacterial rate and specific coefficient of lethality. This test was carried out according to AATCC test method 100-2004.

The circular specimens with 4.8±0.1 cm were prepared and individually placed in a sterile 250 mL Erlenmeyer flask and inoculated with 1 mL bacterial suspensions (10⁷ CFU/mL) and covered with aluminum foil. 100 mL neutralizing solution (sodium thiosulphate (Na₂S₂O₃) 1%, sodium thioglycolate (HSCH₂COONa) 0.6%, and polysorbate 80 0.1% (w/v)) was then added at a proper contact time (0, 0.5, 1, 2, 4, and 6 h) to Erlenmeyer flask containing the inoculated wool fabric sample and then stirred for 30 min at room temperature. The bacterial count was determined by serial dilution and pour plate method

using CASO-Agar medium. The antibacterial efficiency of the loaded wool samples as a term of bactericidal and bacteriostatic were calculated by Eq. (3) and Eq. (4).

$$K\%(\text{Bactericidal} \cdot \text{efficiency}) = \frac{(A - B)}{A} \times 100 \quad (3)$$

$$R\%(\text{Bacteriostatic} \cdot \text{efficiency}) = \frac{(C - B)}{C} \times 100 \quad (4)$$

where A is the number of bacteria recovered from the control sample at 0 contact time and B is the number of bacteria recovered from the treated wool sample at 6 h contact time and C is the number of bacteria recovered from the control sample after 6 h.

Cytotoxicity

a) Morphological changes by microscopy

In vitro cytotoxicity of the treated wool sample with AgNPs entrapped in lecithin was carried out according to ISO-10993-5. 2×5 cm² of wool samples were soaked in 10 mL sterile distilled water for 48 h at 37°C. Mouse fibroblast cells L929 were used as a test model. The cells were cultured in RPMI-1640 which is a medium used in cell and tissue culture and was developed at Roswell Park Memorial Institute (RPMI). This medium was supplemented with 10% bovine fetal serum, 100 IU/mL penicillin and 100 µg/mL streptomycin. An initial density of 1×10⁴ cells/well were seeded in a 96-well tissue culture plate and incubated at 37°C and 5% CO₂ for 48 h. The culture medium of the test wells was then replaced with fresh media containing the extraction solution of the treated wool fabric in three different concentrations (10, 20, and 30 µL). Three replicates were used for the test samples, and four wells were kept as negative control. The plates were kept in an incubator for 72 h. The morphology of the fibroblast structure for various samples was examined by optical microscopy.

b) Measuring cell viability by colorimetric assay

The cells were seeded in a 96-well plate with 1×10⁴ cells/well for the colorimetric assay for 48 h. The attached cells were incubated for 24 h more with colloidal AgNPs (12 ppm) and various lecithin (K_{Ag/ec}= 0, 0.02, 0.2, 0.5, 1 and 2) also RPMI-1640 was used as a control. The cells were then incubated with 25 µl MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide] of 4 mg/mL at 37°C for 3 h. The Formazan crystals were dissolved in DMSO, and the plates were read in a microplate reader (SUNRISE TECAN, Austria) at 540 nm against 620 nm. This experiment was performed in

triplicate and the mean values for each treatment was determined.

RESULTS AND DISCUSSION

Surface Morphology and Chemistry of the Loaded Fabric

The SEM image of the wool samples is presented in *Figure 1*. The characteristic of the wool fibers is scales overlapping structure on the surface (*Figure 1a*) with distinguishable edges. There is a distinctive difference between the stabilized AgNPs with various lecithin as well as applied methods. The pad-dry-cure method produces a fabric with more nano particles deposited on its surface with lower diffusion into the wool fibers. Therefore, the smooth surface scales of the wool fibers were altered with loading of AgNPs. Silver nanoparticles agglomerated at a lower lecithin membrane concentration (*Figure 1b*) due to their high reactivity [14]. However, AgNPs embedded in the bilayer of lecithin structure at high lecithin to the silver ratio (*Figure 1c*). There are few AgNPs on the surface of the loaded wool fiber through the exhaustion method (*Figure 1d and 1e*). This might be due to the carrier role of the lecithin structure as a result of lecithin affinity towards the cell membrane complex of the wool fibers [15].

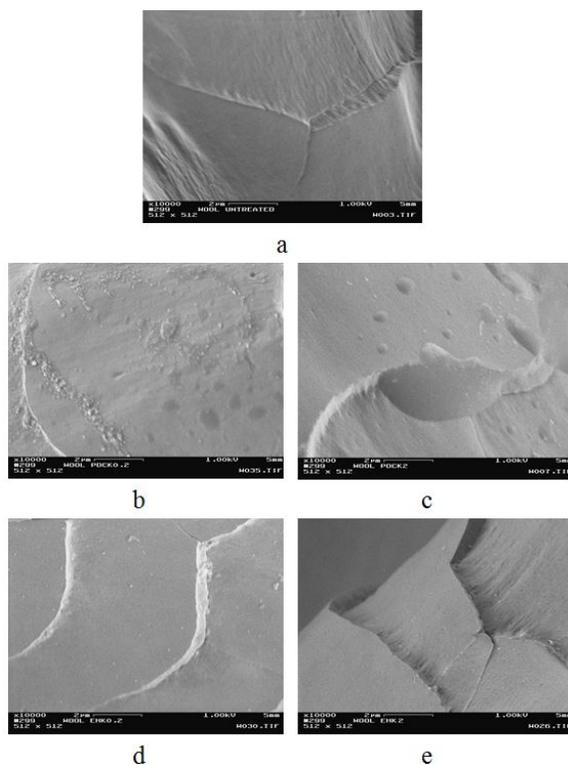


FIGURE 1. SEM image of wool samples a) untreated, b) Loaded with pad-dry-cure and $K_{lec/Ag}=0.2$, c) Loaded with pad-dry-cure and $K_{lec/Ag}=2$, d) Loaded with exhaustion and $K_{lec/Ag}=0.2$, and e) Loaded with exhaustion and $K_{lec/Ag}=2$.

Fourier transform infrared with attenuated total internal reflectance mode analysis were employed to examine the chemical composition of wool fiber surface into 500 nm depth to confirm the presence of AgNPs/lecithin on the surface of wool fiber [17]. ATR-FTIR spectra of the untreated and treated wool fabrics are shown in *Figure 2*. The ATR-FTIR spectra of untreated wool fabric indicate the characteristic bands of proteins at 1635 cm^{-1} , 1518 cm^{-1} , and 1235 cm^{-1} associated to amide I, II, III, respectively [17]. The treated wool with AgNPs/lecithin through pad-dry-cure method presented a lower transmittance intensity compare to the loaded wool through exhaustion method as well as the untreated sample. Thus the exhaustion method leads to the deposition of AgNPs into the wool fiber with a low effect on the surface functional groups. These results are in agreement with the microscopic pictures. The schematic representation of the AgNPs formation into the wool fiber by these two methods is presented in *Figure 3*.

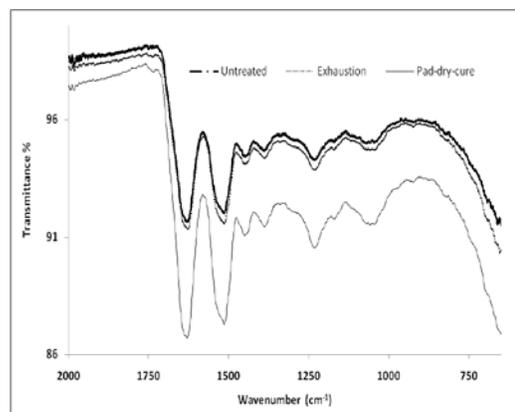


FIGURE 2. ATR-FTIR spectra of untreated and loaded wool fabric with AgNPs/lecithin by pad-dry-cure and exhaustion methods.

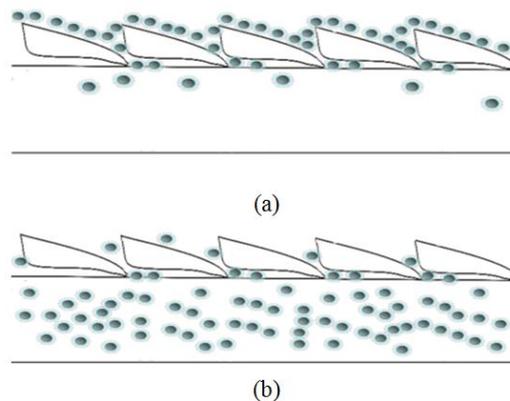


FIGURE 3. Schematic representation of loading AgNPs into the wool fiber by a) pad-dry-cure and b) exhaustion methods.

Silver Content of the Loaded Fabric

The silver content of the loaded wool fabrics is demonstrated in *Table I*. A higher silver content can be seen on the fabrics treated with exhaustion than pad-dry-cure method. More lecithin leads to the higher silver content due to its affinity to the cell membrane complex of wool fibers [15, 18]. The LVSEM pictures indicate that AgNPs deposited on the wool fiber surface through pad-dry-cure while transferred inside the wool fibers through the exhaustion method.

TABLE I. Silver content of loaded sample with different methods in the presence of various lecithin concentrations.

Loading Method	$K_{lec/Ag}=[Lecithin]/[A]$ g]	Silver concentration mg_{Ag}/Kg_{wool}
Exhaustion	0	890
	0.2	1088
	1	3722
	2	3632
Pad-Dry-Cure	0	72
	0.2	616
	1	680
	2	948

Color Measurement

The color strength (K/S) of all loaded fabrics with different methods and lecithin concentrations are presented in *Figure 4*. The loaded fabrics show higher color strength than the untreated one due to the AgNPs loading. The amount of the deposited AgNPs on the fabric is related to K/S value [16]. The K/S is the ratio between the absorption (K) and scattering coefficient (S) of the coated substrate. The K/S of different treated wool fabrics by exhaustion method indicates higher color strength than pad-dry-cure method. Thus, the AgNPs loading in exhaustion was higher than pad-dry-cure in accord with the ICP-OES results. However, it is difficult to find a relation between the color strength of the fabric with different lecithin and the loading efficiency as AgNPs absorption significantly depends upon the shape, size and dielectric media [19]. However, increasing lecithin is an influencing factor in the absorption of the AgNPs into the wool fibers.

Figure 5 displays the yellowness indexes of the untreated and treated fabrics through different methods and lecithin concentrations. The yellowness indexes are measured primarily to study the yellowing effect of the processing on the fabric. Higher lecithin concentration leads to the more loading efficiency as well as lower yellowness indexes through different methods. This can be due to

the nano silvers entrapment within the lecithin layers that prevented the AgNPs oxidation and reduced yellowing.

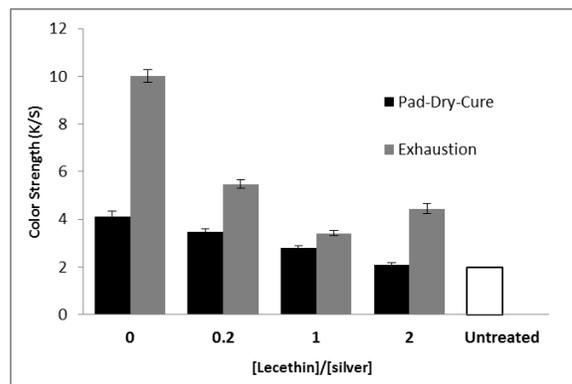


FIGURE 4. Color strength of the loaded samples with different methods and various lecithin concentrations.

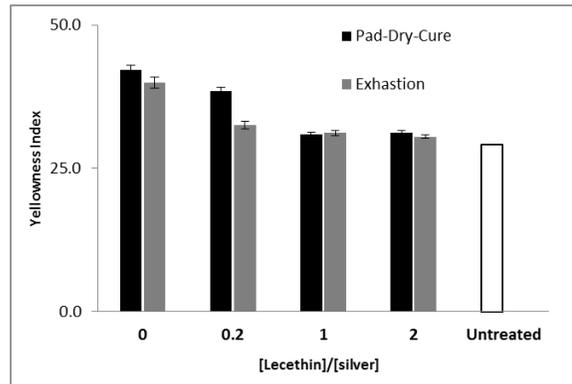


FIGURE 5. Yellowness index of the loaded samples with different methods and various lecithin concentrations.

Antibacterial Properties of the AgNPs Loaded Wool Fabric

The antibacterial properties of the AgNPs loaded wool fabrics were also investigated against a Gram-positive and a Gram-negative bacterium. *Table II* shows the inhibition zone of the wool samples produced through two different methods. The fabrics with the most silver content show the larger inhibition zone, as the antibacterial activities depends on the silver content [20, 21]. This should be higher than the minimum inhibitory concentration (MIC) of the silver [13]. The loaded fabrics through exhaustion with $K_{lec/Ag} \geq 1$ and pad-dry-cure, $K_{lec/Ag} = 2$ indicate the antibacterial activity due to the higher loading efficiency and stabilizing role of lecithin. While, other loaded fabrics with lower lecithin concentration have not presented inhibition zones due to the lower loading efficiency as well as instability of the synthesized AgNPs. It has been reported that silver nanoparticles solution with low lecithin easily

agglomerated and no antibacterial activities indicated [13].

TABLE II. Mean value of inhibition zone diameter (mm) of AgNPs loaded wool fabric with various lecithin ratios.

Bacterial Strain	Exhaustion				Pad-dry-cure			
	K ₀	K _{0.2}	K ₁	K ₂	K ₀	K _{0.2}	K ₁	K ₂
<i>S. aureus</i>	--- ^a	---	1	1	---	---	---	0.75
<i>E. coli</i>	---	---	0.75	0.75	---	---	---	0.75

^aNo inhibition zone width.

The K₀, K_{0.2}, K₁, and K₂ are presented the [Lecithin]/[Ag]=0, 0.2, 1, and 2, respectively. Experiments were performed in duplicates

The bactericidal and bacteriostatic efficiency of the loaded wool fabrics with different lecithin ratios as a stabilizing agent are shown in *Table III* and *Table IV*. All the exhausted loaded wool fabrics show antibacterial activities due to the antibacterial properties of the silver ions as well as AgNPs [22]. In contrast to the qualitative test, the unstabilized AgNPs release the silver ions in the presence of the aqueous solution. Thus, the treated wool fabric indicated the antibacterial activities in the moist condition.

TABLE III. Antibacterial activity of loaded wool fabrics against *E. coli*.

Efficiency	Exhaustion				Pad-dry-cure			
	K ₀	K _{0.2}	K ₁	K ₂	K ₀	K _{0.2}	K ₁	K ₂
K %	43.5	69.3	85.2	90.6	---	---	---	59.3
R %	90.9	95.0	96.7	98.5	---	---	---	93.4

The bacteriostatic efficiency (R%) of all loaded fabrics by exhaustion method is higher than 90%. Moreover, the bactericidal efficiency (K%) of the loaded fabric with K_{lec/Ag}=2 against *E. coli* and *S. aureus* were 90.6% and 94.2%, respectively in exhaustion method. However, 78% bacteriostatic efficiency for the loaded fabric with silver/chitosan was reported [22]. Thus, lecithin led to the improved stability and loading efficiency of the AgNPs and remarkably increased the antibacterial properties. All loaded wool fabrics presented a high antibacterial activity against *S. aureus* than *E. coli* as reported in the previous paper [23].

TABLE IV. Antibacterial activity of the loaded wool fabrics against *S. aureus*.

Efficiency	Exhaustion				Pad-dry-cure			
	K ₀	K _{0.2}	K ₁	K ₂	K ₀	K _{0.2}	K ₁	K ₂
K %	87.5	90.0	91.1	94.2	---	---	56.7	90.6
R %	91.1	96.9	97.2	98.8	40.5	44.4	86.6	97.1

The bactericidal and bacteriostatic efficiencies of the loaded wool fabrics after washing are presented in *Table V* and *Table VI* and indicate a lower antibacterial efficiency after washing. The loaded

fabrics through exhaustion showed a higher wash fastness than pad-dry-cure. The exhaustion process led to the better diffusion and entrapment of the AgNPs inside the wool fiber structure [24] improved the wash fastness. However, the AgNPs through the pad-dry-cure adsorbed on the fiber surface and was easily removed by washing.

TABLE V. Antibacterial activity of the loaded wool fabrics against *E. coli* after washing.

Efficiency	Exhaustion				Pad-dry-cure			
	K ₀	K _{0.2}	K ₁	K ₂	K ₀	K _{0.2}	K ₁	K ₂
K %	---	10.2	36.1	59.2	---	---	---	---
R %	18.1	58.7	80.2	87.3	---	---	---	61.2

TABLE VI. Antibacterial activity of the loaded wool fabrics against *S. aureus* after washing.

Efficiency	Exhaustion				Pad-dry-cure			
	K ₀	K _{0.2}	K ₁	K ₂	K ₀	K _{0.2}	K ₁	K ₂
K %	---	44.2	57.8	82.6	---	---	---	48.2
R %	73.0	91.0	93.2	97.2	---	---	26.2	78.3

The specific coefficient of lethality can be determined by using Chick-Watson equation [25]:

$$\frac{dN}{dt} = -KC \quad (5)$$

$$\ln \frac{N}{N_0} = -Kct \quad (6)$$

N is the number of CFU per milliliter of solution at the time t, N₀ is the initial number of CFU per milliliter of solution at the beginning of the test, C is the concentration of synthesis AgNPs on wool fabric and K is the specific coefficient of lethality. *Figure 6* shows the specific coefficient of lethality of the treated wool fabric with the colloidal AgNPs solution on *S. aureus* and *E. coli* that was obtained by adopting the bacterial inactivation data to Chick-Watson model through linear regression. Increasing lecithin concentration reduced the killing rate and specific coefficient of lethality. Therefore, a high lecithin concentration led to the higher loading efficiency and antibacterial activities [21]. However, lecithin molecules form a membrane bilayer structure that entrapped the AgNPs within layers and reduced the release rate of the silver ions and nanoparticles finally resulting in a lower killing rate.

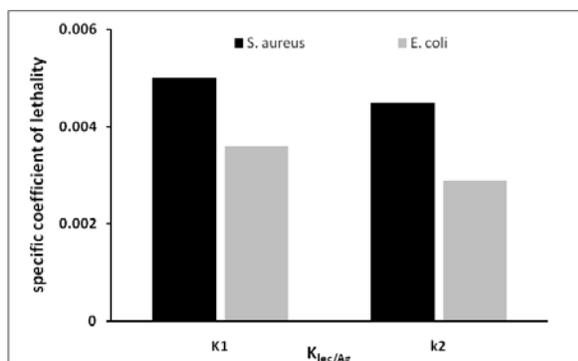


FIGURE 6. The specific coefficient of lethality of loaded wool samples with various lecithin concentrations. The K_1 and K_2 are presented the $[Lecithin]/[Ag]=1$ and 2, respectively.

Cytotoxicity

The cytotoxicity of the extracted solution of the loaded wool fabric on morphology of L929 cells is shown in *Figure 7* obtained by optical microscopy after three days. This indicates the changes in the cell morphology due to their exposure to the toxic materials [27]. The morphology of the cultured fibroblast cells in the presence of the extracted solutions of the loaded wool fabric with different lecithin concentration was not changed and is similar to the morphology of the control sample.

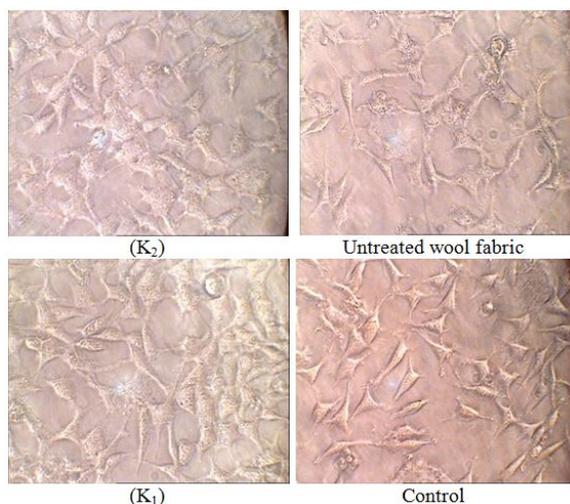


FIGURE 7. Morphology of L929 cell culture in presence of extraction solution of wool loaded fabric with AgNPs and a control sample after 72 hours.

The extracted solutions have no influences on the morphology of the cell lines; however this is not enough to judge on the cell viability. The results of MTT assay which provide the viability of the cell line in the presence of the colloidal silver nanoparticle

solution (12.5 ppm) is presented in *Figure 8*. More lecithin, as a safe biological material, lead to the improved cell viability and protected the cell lines from AgNPs. These results are in accord with the reduced cytotoxicity of polyhexamethylene biguanide hydrochloride (PHMB) by lecithin with good antimicrobial efficiency [26]. Overall, AgNPs entrapment within lecithin layers prepares a nontoxic antibacterial agent with reasonable activity.

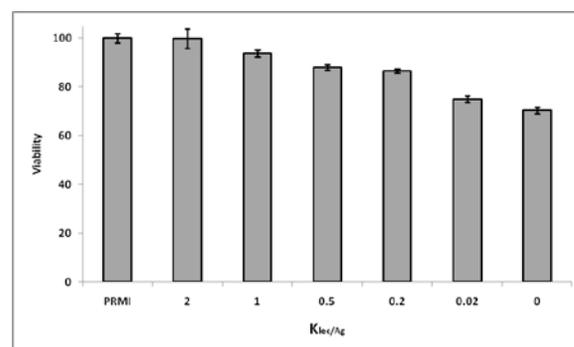


FIGURE 8. The cell viability of fibroblast in the presence of colloidal AgNPs solutions which stabilized by different lecithin concentrations.

CONCLUSION

This research examined the role of lecithin as a biological surfactant in the adsorption of AgNPs within the wool fiber structure and confirms the influences of lecithin concentration and application methods on the loading efficiency of AgNPs as well as washing fastness of the loaded wool fabric. The fabrics treated through pad-dry-cure method indicate the deposition of AgNPs on the wool surfaces, while the exhausted fabrics show the diffusion of Ag NPs within the wool fibers. The LVSEM images confirm deposition of the AgNPs on the wool fiber surface. However, more lecithin improved the loading efficiency of nano silver on the wool fabrics due to the carrier role of lecithin. There is no correlation between the color strength and the silver content of the loaded fabrics with different lecithin concentrations due to the different synthesis conditions. Further, the antibacterial activities of the loaded wool fabrics related to the silver content as the loaded fabric with a high lecithin to silver ratio ($K_{Lec/Ag}=2$) indicate a larger zone of inhibition and higher loading efficiencies. Further, the cell viability of the fibroblast cells indicate the influence of the lecithin concentration on protecting the cell lines with presence of AgNPs at high lecithin concentration that leads to synthesis of a non-toxic antibacterial agent.

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AUTHORS' ADDRESSES

Hossein Barani

Majid Montazer

Nasrin Samadi

Tayebeh Toliyat

Mohsen Khorashadi Zadeh

Boudewijn de Smeth

Amirkabir University of Technology

Hafez Avenue

Tehran, Tehran15875-4413

IRAN