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Photo induced silver on nano titanium dioxide as an enhanced antimicrobial agent for wool

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ABSTRACT

In this study an effective nanocomposite antimicrobial agent for wool fabric was introduced. The silver loaded nano TiO_2 as a nanocomposite was prepared through UV irradiation in an ultrasonic bath. The nanocomposite was stabilized on the wool fabric surface by using citric acid as a friendly cross-linking agent. The treated wool fabrics indicated an antimicrobial activity against both *Staphylococcus aureus* and *Escherichia coli* bacteria. Increasing the concentration of Ag/TiO_2 nanocomposite led to an improvement in antibacterial activities of the treated fabrics. Also increasing the amount of citric acid improved the adsorption of Ag/TiO_2 on the wool fabric surface leading to enhance antibacterial activity. The EDS spectrum, SEM images, and XRD patterns was studied to confirm the presence of existence of nanocomposite on the fabric surface. The role of both cross-linking agent and nanocomposite concentrations on the results was investigated using response surface methodology (RSM).

1. Introduction

Population explosion and environmental pollution encourage researchers to seek hygienic and secure production methods to facilitate human living conditions [1]. In recent decades, there have been a lot of developments using nanotechnology in the textile industry. For instance, different kinds of antimicrobial treatments have been utilized to protect garments against harmful microorganisms [2,3]. Among various nanoparticles, TiO_2 has attracted a great deal of scientists' attention because of its chemical stability, low cost, ease of availability, non-toxicity, and optical properties [4,5].

The photocatalytic features of TiO_2 under UV rays have been confirmed. When TiO_2 is irradiated by UV rays ($\lambda < 388$ nm), an electron from the valence band is promoted to the conduction band, producing pairs of negative electrons (e^-) and positive holes (h^+) [5–8]. These active species play an important role in initiating oxidation and reduction reactions [8]. Although there are numerous advantages in utilizing the TiO_2, there are some disadvantages for the pure one as follows. Firstly, nano TiO_2 shows the photocatalytic activities just under UV rays which is one of the major barriers in developing its usage and secondly the electron–hole recombination rate is too high resulting in low photocatalytic efficiency [9]. It has been suggested that through adding noble metals on the surface of TiO_2 its demerits can be reduced [10,11]. This phenomenon occurs through mitigating the rate of electrons and holes recombination by trapping the electrons, expanding the light efficiency into visible territory, and modifying the surface properties of photocatalysts [10]. Some noble metals such as Pt [12,13], Au [14,15], and Ag [10] have been used for this purpose. It has been confirmed that through modifying the TiO_2 nanoparticles by silver ions photoactivity and antimicrobial features are improved [10]. In addition, Ag/TiO_2 nanocomposite shows antibacterial activities even under visible rays [10,16].

Some pieces of research have been conducted regarding the application of nanoparticles on textiles. For instance, Daoud and Qi obtained self-cleaning keratins and cottons through the sol–gel method [17,18]. Along the same lines, Montazer and Pakdel reduced the photoyellowing of wool by TiO_2; they also obtained self-cleaning wool fabrics through applying the nano TiO_2 particles immobilized by carboxylic acids [19–21]. Moreover, Bozzi et al. produced the self-cleaning wool–polyamide fabric using nano TiO_2 stabilized by plasma pretreatment [22]. Some studies have focused on imparting the antimicrobial properties to textiles by

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employing various substances. For example, Wang obtained antimicrobial wool using nano SiO_2 [23]. Singh et al. assessed the antimicrobial property of some natural dyes on wool [23,24]. Additionally, other compounds such as quaternary ammonium salts [25], chitosan [26], and curcumin [27] have been employed to produce the antimicrobial fabrics.

In this study, the nanocomposites were anchored on the surface of wool using citric acid. It has been confirmed that nano TiO_2 particles in acidic solutions have positive charges leading to a great tendency towards carboxyl and hydroxyl groups with negative charges [17–21]. Also citric acid as a cross-linking agent introduces additional negative charged groups on the wool surface increasing the nanoparticles adsorption [19].

Lack of scientific reports on antibacterial activities of Ag/TiO_2 nanocomposites on textiles, encouraged the researchers of this study to assess the antibacterial activity of treated wool fabrics with Ag/TiO_2 against two common pathogenic bacteria: *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*). The antibacterial studies were conducted through two diverse methods of biofilm and suspension. Bacterium biofilm was done on the fabric surface as a qualitative method and bacterium growth measurement was carried out by suspension as a quantitative method. In order to conduct an accurate investigation, response surface methodology (RSM) was used through which the impact of each independent variable on response surfaces was evaluated. RSM allows model adequacy including lack of fit, designs of higher order to be built up sequentially, and provides an internal estimate of error [20]. Furthermore, response surface designs neither require a large number of runs nor too many levels of independent variables [28,29].

2. Materials and methods

2.1. Materials

A 100% wool fabric with twill structure, and 159 g/m^2 fabric mass was used. Nano titanium dioxide powder (Degussa P-25) with average particle size of about 21 nm from Evonik (Germany) and silver nitrate with 99% purity from Merck (Germany) were employed to prepare the nanocomposite. Citric acid (CA) and sodium hypophosphite (SHP) with purity of more than 99% were purchased from Merck (Germany). The investigated microorganisms were *E. coli* (ATCC 11303) and *S. aureus* (ATCC 1112). Tryptic soy agar culture medium was bought from Merck (Germany).

2.2. Apparatus

A UV-A bulb (400 W, HPA, 320 nm < λ < 400 nm) from Philips (Belgium) was used as an artificial UV source. XRD patterns were obtained using Philips X' Pert MPD diffractometer with a Cu generator bulb (40 KV, 40 mA) (The Netherlands). SEM images and EDS spectra were obtained using XL30 scanning electron microscopy (Netherlands). An ultrasonic bath model DSA 100-XN1-2.5L, 100 W, 40 kHz (Taiwan) was used to mix the components of the impregnating bath. In addition, to create linkages between wool and carboxylic acid (CA) treated samples were cured in an oven.

2.3. Ag/TiO_2 nanocomposite preparation

To produce Ag/TiO_2 nanocomposite, first TiO_2 along with 100 mL distilled water was poured into a volumetric flask in the required amount. Next, in order to suspend the nano TiO_2 particles in water the solution was sonicated in the ultrasonic bath for 10 min. Subsequently, silver nitrate was added to the solution and then the finishing bath was illuminated by UV-A rays for 15 min. Finally, the nanocomposite can be obtained with turning the color solution into cream-brown.

Ag/TiO_2 nanocomposite is produced as a result of the reaction between silver nitrate and titanium dioxide in the ultrasonic bath under UV rays. The reactions between TiO_2 and $AgNO_3$ are summarized in [30–32].

$$AgNO_3 \xrightarrow{UV,US} Ag_2O + NO_2 + NO \quad (1)$$

$$Ag_2O \xrightarrow{UV,US} Ag + O_2 \quad (2)$$

$$Ag + TiO_2 \xrightarrow{UV,US} Ag/TiO_2 \quad (3)$$

2.4. Scouring

The wool samples were washed in a bath containing 2 g/L nonionic detergent with L:G = 50:1 (liquor to good ratio) at 50° C for 15 min. Then, it was rinsed with distilled water and dried at 110 °C for 5 min.

2.5. Ag/TiO_2 nanocomposite treatment

Diverse amounts of Silver/titanium dioxide nanocomposite, citric acid (CA), and sodium hypophosphite (SHP) were used in the finishing bath with 100 mL volume according to Table 1. The prepared solution was sonicated for 10 min and then the samples were put into the bath and maintained for 30 min. The treated samples were dried at 110 °C for 10 min and then cured at 160 °C for 4 min.

2.6. Antibacterial test using biofilm method (qualitative method)

In this method, through a sterile clip several pieces of fabric were placed in separate test tubes containing 0.5 mL Muller-Hinton broth and then 0.5 mL of bacteria culture was added to each tubes. After a slight shake, the tubes were put in an incubator at 37 °C for 18–24 h. Subsequently, using a sterile clip, a piece of fabric was taken out from each test tube and washed with distilled water. The washed samples were then put in the agar Muller-Hinton media for 1 min transferring the bacteria from the samples into the agar. Afterwards, the pieces of fabric were picked up using a sterile clip and then the culture medium was incubated at 37 °C for 18 h. After the incubation, the growth of bacteria colonies was compared visually [33,34].

2.7. Antibacterial test using suspension method (quantitative method)

Two pathogenic microorganisms including *E. coli* (gram negative) and *S. aureus* (gram positive), as two most evaluated species were tested by using AATCC 100-2004 test method. The number of viable bacteria colonies on the agar plate before and after a nanocomposite treatment was counted and the results reported as percentages of bacteria reduction according to

$$\text{Bacteria reduction } (R)\% = 100(A - B)/A \quad (4)$$

where (A) and (B) are the numbers of bacteria colonies recovered from the untreated and the treated wool samples respectively after inoculation and incubation and (R) is the reduction percentage of bacteria colonies [35].

Table 1
Results and experimental situation base on CCD.

Run	TiO_2 (mg/L)	$AgNO_3$ (mg/L)	CA (mg/L)	SHP (mg/L)	Antibacterial activity against *S. aureus* (Y_1)	Antibacterial activity against *E. coli* (Y_2)
1	1.01×10^4	1.01×10^2	9.60×10^2	5.76×10^2	99.81	100
2	4.10×10^3	41	1.54×10^2	9.24×10^2	0	0
3	1.60×10^4	1.60×10^2	4.00×10^2	2.40×10^2	99.26	96.45
4	1.60×10^4	1.60×10^2	4.00×10^2	9.24×10^2	99.88	100
5	2.01×10^4	2.01×10^2	9.60×10^2	5.76×10^2	100	100
6	1.01×10^4	1.01×10^2	9.60×10^2	1.15×10^2	99.25	100
7	4.10×10^3	41	4.00×10^2	2.40×10^2	0	0
8	1.01×10^4	1.01×10^2	9.60×10^2	5.76×10^2	99.81	100
9	1.01×10^4	1.01×10^2	9.60×10^2	5.76×10^2	99.81	100
10	0	0	9.60×10^2	5.76×10^2	13.88	0
11	1.01×10^4	1.01×10^2	9.60×10^2	5.76×10^2	81.99	100
12	1.01×10^4	1.01×10^2	0	5.76×10^2	56.85	95.96
13	4.10×10^3	41	1.54×10^2	2.40×10^2	0	0
14	1.60×10^4	1.60×10^2	1.54×10^2	2.40×10^2	99.63	100
15	4.10×10^3	41	4.00×10^2	9.24×10^2	34.81	0
16	1.60×10^4	1.60×10^2	1.54×10^2	9.24×10^2	100	100
17	1.01×10^4	1.01×10^2	9.60×10^2	5.76×10^2	99.81	100
18	1.00×10^4	1.00×10^2	9.60×10^2	0	98.33	99.52
19	1.01×10^4	1.01×10^2	1.90×10^3	5.76×10^2	99.07	100
20	1.01×10^4	1.01×10^2	9.60×10^2	5.76×10^2	99.81	100

3. Results and discussions

3.1. Antimicrobial activity of treated samples

After the specified contact time, antimicrobial activities of raw and the treated samples against both *E. coli* and *S. aureus* bacteria were calculated. The *S. aureus* bacterium is a pathogenic microorganism causing many diseases such as toxic shock, purulence, abscess, fibrin coagulation, and endocarditic. Moreover, it is resistant to common antimicrobial agents [36]. Furthermore, *E. coli* bacterium which causes urinary tract and wound infections is a popular test organism [27].

It has been confirmed that through adding metal ions such as Ag to the surface of TiO_2, the photocatalytic activities of TiO_2 can be enhanced due to the lower recombination rate of generated negative electrons and positive holes [10]. In this situation, antimicrobial property of TiO_2 is one of the main parameters that can be improved. Absorbing higher amounts of nanoparticles on the surface of fabrics creates enhanced novel features. For example, through increasing the concentration of citric acid as a cross-linking agent in the impregnating bath more suitable sites are prepared on the surface of wool leading to a higher adsorption of Ag/TiO_2 and consequently higher antimicrobial property. Likewise, a higher amount of nanocomposite in the impregnating bath provided a better antimicrobial property due to the higher deposition of nanoparticles on the surface of wool.

Fig. 1 indicates that adding Ag ions to nano TiO_2 particles had tangible effect on its antimicrobial activity and its efficiency was higher than either Ag or TiO_2. Also, it was observed that using the highest amount of nanocomposite (Ag/TiO_2) and cross-linking agent (CA) in the impregnating bath could provide the best antimicrobial activity. A suggested mechanism that explains the inhibition of microorganisms is that obtained nanocomposite under UV rays generates some positive species that react with negatively charged residues at the cell surface of microorganisms, causing an alteration of cell permeability, as a result of which the normal metabolism of microorganisms changes and they are therefore eliminated [37]. It has been mentioned that generated oxygen species (O_2^-) can oxidize organic compounds of bacteria cells [38,39]. Fig. 1 shows the antimicrobial activity of untreated wool sample (blank) and treated ones in different conditions according to the biofilm method.

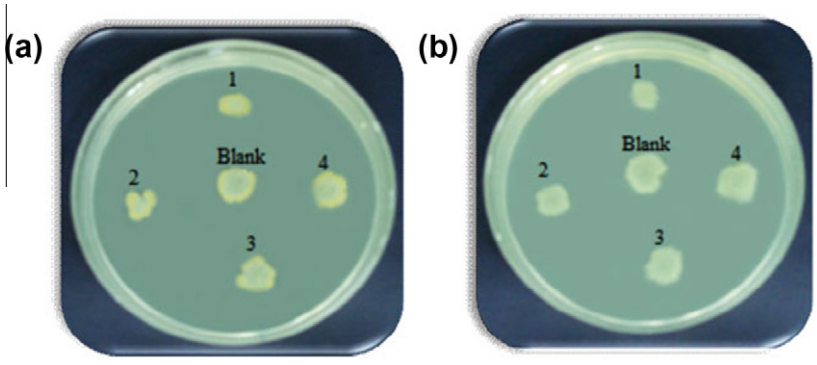

Fig. 1. Bacterial growth on the blank and treated samples against: (a) *S. aureus* and (b) *E. coli*. (1) 2×10^4 mg/L Ag/TiO_2 nanocomposite 1.9×10^3 mg/L CA, 1.54×10^3 mg/L SHP. (2) 2×10^4 mg/L nano TiO_2, 1.9×10^3 mg/L CA, 1.54×10^3 mg/L SHP. (3) 2×10^2 mg/L $AgNO_3$ 1.9×10^3 mg/L CA, 1.54×10^3 mg/L SHP. (4) 1.9×10^3 mg/L CA, 1.54×10^3 mg/L SHP.

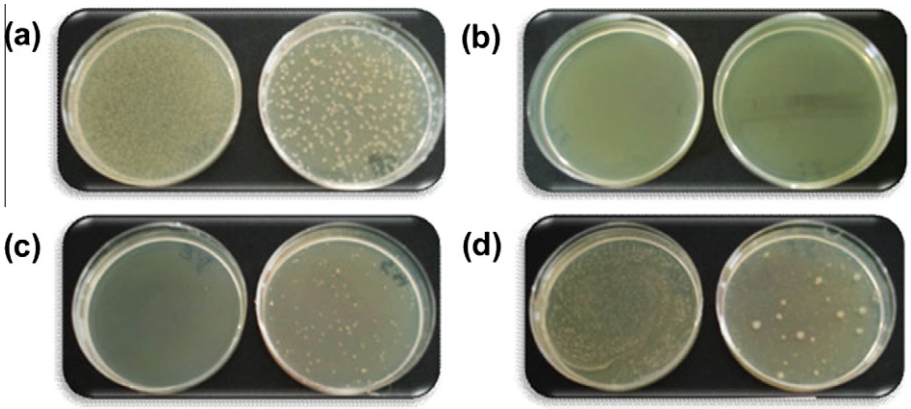

Fig. 2. Antibacterial wool fabric based on quantitative method: *S. aureus* (right) and *E. coli* (left) treated with (a) blank sample, (b) 2×10^4 mg/L Ag/TiO_2 nanocomposite, 1.9×10^3 mg/L CA and 1.54×10^3 mg/L SHP, (c) TiO_2 nano particles, 1.9×10^3 mg/L CA, 1.54×10^3 mg/L SHP, and (d) 2×10^4 mg/L TiO_2, 2×10^2 mg/L $AgNO_3$, 1.9×10^3 mg/L CA, 1.54×10^3 mg/L SHP.

Fig. 1 indicates that the treated sample with Ag/TiO_2 nanocomposite, CA, and SHP has a higher antibacterial activity against both *S. aureus* and *E. coli* bacteria. Hence, the nanocomposite has been more successful in inhibiting the growth of bacteria. In fact, the treated sample with TiO_2, CA, and SHP shows lower antibacterial activities. Fig. 2 reveals the higher antimicrobial properties on the nanocomposite treated fabrics in comparison with other samples. It was observed that the treated sample with Ag/TiO_2 along with cross-linking agent in comparison with treated fabric with TiO_2 alone and also the treated fabric without cross-linking agent had a higher antimicrobial activity. In Fig. 2a it can be seen that the growth of bacteria on the blank sample whereas the growth of the bacteria was inhibited by Ag/TiO_2 treated fabric (Fig. 2b). In other words, the raw wool sample demonstrates no antibacterial activity against both *E. coli* and *S. aureus* microorganisms (Fig. 2b).

Montazer and Pakdel demonstrated that through increasing the amount of nanoparticles in the impregnating bath novel features appeared with a better quality [19–21]. Similarly, it was observed that through adding the nanocomposite in the finishing bath antibacterial property increased in comparison with the untreated wool. Through increasing the nanocomposite concentration a slight improvement in the antimicrobial activity of treated samples was observed (Fig. 3). Ag/TiO_2 was efficient to provide antimicrobial property even in low concentrations. Each factor that gives rise to a better adsorption of nanoparticles on the surface of wool leads to better photocatalytic activities. In addition, citric acid is an essential cross-linking agent because of its ability in creating ester linkages between functional groups of wool fabric and carboxylic groups of citric acid. It was observed that increasing the concentration of CA and SHP causes a better antimicrobial activity, providing more sites for creation of the linkages. These results are consistent with the previous findings [20]. It was also confirmed that the presence of metal ions such as Ag on the TiO_2 led to a considerable improvement in the antimicrobial activity particularly in lower concentrations of nanoparticles. Ag ions increases the separation rate of photo-induced negative electrons and positive holes, providing a better photocatalytic property (Figs. 3 and 4).

Based on the obtained results, antimicrobial agents showed a better efficiency against *E. coli* in comparison with *S. aureus*. This can be explained by the difference between thicknesses of the cell walls. *S. aureus* has a thicker cell wall; therefore, the reactions between active species and suitable sites of *S. aureus* are not as efficient as that of *E. coli* [38,39]. The features of treated samples with nano TiO_2 alone are strongly dependent upon the amount of citric acid and sodium hypophosphite and decreasing the amount of citric acid and sodium hypophosphite will decrease the antibacterial activities substantially. However, this is not similar for the treated samples with Ag/TiO_2, since this nanocomposite show a suitable antibacterial activity even in the absence of citric acid and sodium hypophosphite. In addition, one drawback of silver nitrate is creation of brown patches on the samples surface after irradiating. Nevertheless, this problem does not occur for

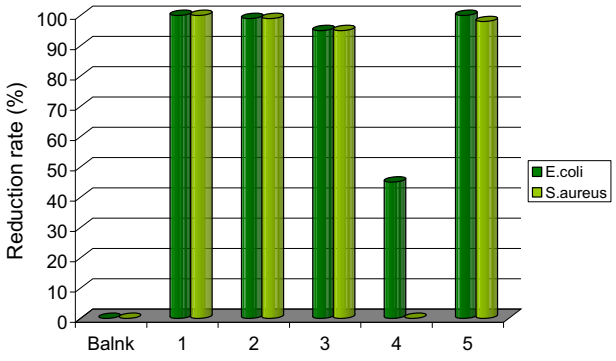

Fig. 3. The impacts of nano particles and concentration on the antimicrobial activity against two microorganisms on the treated samples with nano particles, 1.9×10^3 mg/L CA and 1.54×10^3 mg/L SHP. (1) 2×10^4 mg/L Ag/TiO_2. (2) 5×10^3 mg/L Ag/TiO_2. (3) 2×10^4 mg/L TiO_2. (4) 5×10^3 mg/L TiO_2. (5) 2×10^2 mg/L $AgNO_3$.

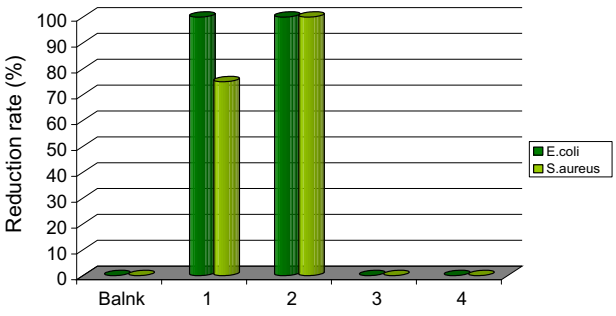

Fig. 4. The role of cross-linking agent on the reduction rate of microorganisms on the blank and treated samples with: (1) 2×10^4 mg/L Ag/TiO_2 nanocomposite without cross-linking agent. (2) 2×10^4 mg/L Ag/TiO_2 nanocomposite, 9.6×10^2 mg/L CA and 5.76×10^2 mg/L SHP. (3) 2×10^4 mg/L TiO_2 without cross-linking agent. (4) 2×10^2 mg/L $AgNO_3$ without cross-linking agent.

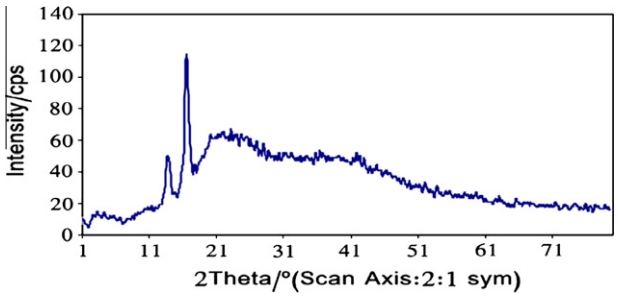

Fig. 5. XRD pattern of raw wool.

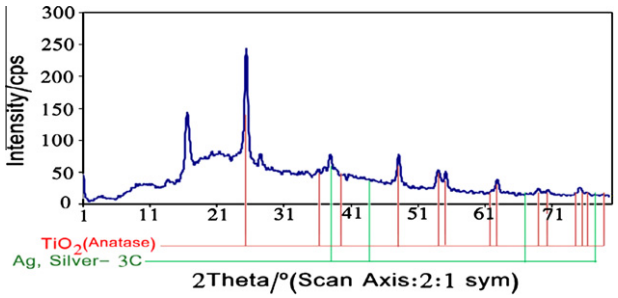

Fig. 6. XRD pattern of treated wool with Ag/TiO_2, CA and SHP.

the samples treated with Ag/TiO_2 nanocomposite or TiO_2 nanoparticles. The produced nanocomposite provides a higher antibacterial property in comparison with silver nitrate or nano titanium dioxide.

3.2. XRD patterns

Analysis of the X-ray diffraction patterns is a suitable method to identify the crystallinity of the samples. Also, it is used to calculate the phase content and investigate the changes of phase structure of the samples before and after the treatment [40]. The XRD patterns of raw and treated wool are shown in Figs. 5 and 6, respectively. Concerning raw wool, two major peaks can be seen around $2\theta = 13.7°$ and $2\theta = 16.45°$ which are related to the hydrated crystalline structure of wool and α-keratin, respectively [40]. In the treated sample, the major peak is around $2\theta = 25.3°$ related to anatase structure of nano TiO_2 applied on the surface of wool and in $2\theta = 37.9°$ the minor peaks of rutile structure of TiO_2 exist. One of the ingredients of the impregnating bath was Ag/TiO_2, as a result of which peaks of Ag appeared around $2\theta = 38°$. These results are consistent with the previous findings [19,32]. Also, through the full width at half maximum of peaks (FWHM), the crystal sizes can be compared [32]. Higher FWHM shows the small crystals while lower FWHM indicates bigger crystals [19]. Based on Eq. (5) the crystal sizes were calculated and for raw wool at $2\theta = 16.45°$ and treated one at $2\theta = 25.3°$ were 129.26 Å and 169.17 Å respectively.

$$\text{Crystals size (Å)} = \frac{K \times \lambda \times 180}{\text{FWHM} \times \pi \times \cos\theta} \quad (5)$$

where $K = 0.9$ is the shape factor, $\lambda = 1.54$ is the wavelength of X-ray of Cu radiation, FWHM is full width at half maximum of the peak, and θ is the diffraction angle [19].

3.3. SEM images

Through the scanning electron microscopy (SEM) images, the morphology of wool samples was studied. Fig. 7a indicates that the raw wool surface is covered with scales overlapping each other. It has been demonstrated that through covering the outer layer of wool fibers by nanoparticles some features can be obtained; for instance, self-cleaning, UV-protection, hydrophilicity among others. Here, the outer layer of wool was covered by Ag/TiO_2 nanocomposites creating an antimicrobial activity on wool samples. Fig. 7b and c shows the surface of the treated samples.

3.4. EDS spectrum

Energy-dispersive X-ray spectrometry (EDS) is an analytical technique used for the elemental analysis or chemical

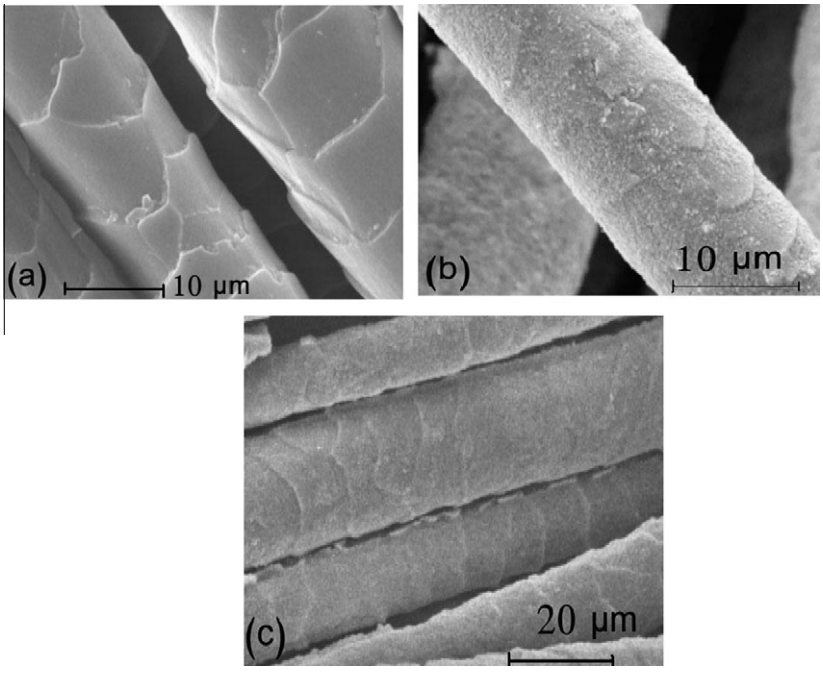

Fig. 7. SEM images of wool samples: (a) raw wool (2000×), (b) treated wool with Ag/TiO_2, CA and SHP (2000×), and (c) treated wool with Ag/TiO_2, CA and SHP (1000×).

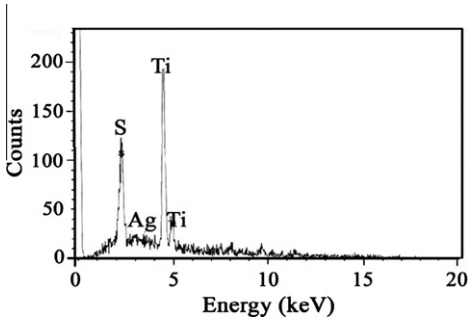

Fig. 8. EDS spectrum of treated sample with Ag/TiO_2, CA and SHP.

characterization of a sample and detect the elements using X-rays that are emitted from the specimen [25]. Energy peaks correspond to the various elements in the sample. The presence of titanium and silver particles on the treated sample surface was proved using EDS. In addition, the existence of some elements such as carbon, sulfur and oxygen were identified (Fig. 8).

3.5. Statistical analysis

This study was conducted according to response surface methodology (RSM) and central composite design (CCD). In total, 20 experimental CCD designed runs were conducted according to Table 1. In this model, the impacts of independent variables including Ag/TiO_2 nanocomposite, citric acid and sodium hypophosphite concentrations in the impregnating bath on two response surfaces were assessed. For each of them optimum condition was obtained (Table 1), through which the variation of two response surfaces including Y_1 and Y_2 was discussed. These explain antimicrobial activity of treated samples against *S. aureus* and *E. coli* bacteria respectively. Based on the results of relations between surface responses and independent factors, several mathematical models were obtained (Eqs. (6) and (7)), whose related responses were a function of its independent variables. The response surfaces are proposed in Fig. 9.

Mathematical models related to antimicrobial activity of treated samples against *S. aureus* and *E. coli* is shown in Eqs. (6) and (7) respectively:

$$Y_1 = -79.21 + 205.61\ (Ag/TiO_2) + 4.34\ (CA) + 6.43\ (SHP) + 0.13\ (Ag/TiO_2)\ (CA) + 0.22\ (Ag/TiO_2)\ (SHP) - 0.02\ (CA)\ (SHP) - 69.03\ (TiO_2/Ag)^2 - 0.21\ (CA)^2 - 0.53\ (SHP)^2 \quad (6)$$

$$Y_2 = -89.23 + 174.98\ (TiO_2/Ag) + 7.42\ (CA) + 9.52\ (SHP) + 1.28\ (Ag/TiO_2)\ (CA) - 2.03\ (Ag/TiO_2)\ (SHP) - 0.19\ (CA)\ (SHP) - 56.26\ (TiO_2/Ag)^2 - 0.35\ (CA)^2 - 0.39\ (SHP)^2 \quad (7)$$

As shown in Fig. 9, increasing the amount of antimicrobial agent resulted in more reduction of both bacteria. In addition, increasing the concentration of CA led to a higher adsorption of nanocomposites on the wool surface. Subsequently, under UV rays higher positive species were produced and reacted with negative residues

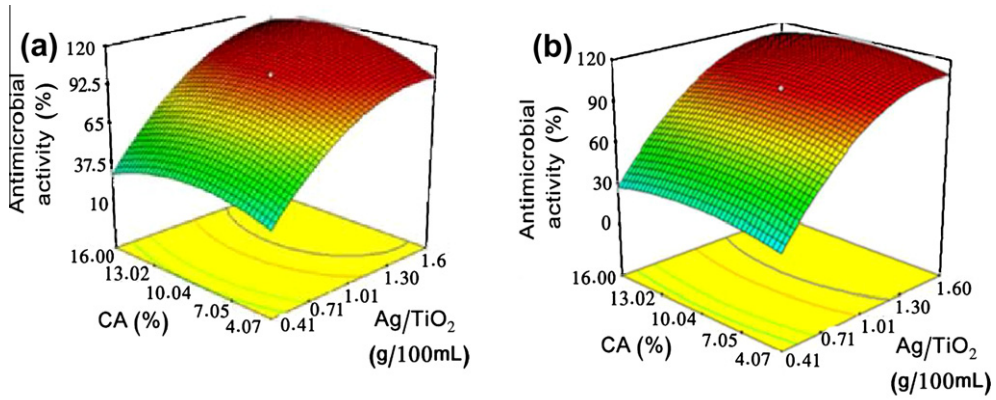

Fig. 9. Response surface for antimicrobial activity as a function of Ag/TiO_2 and CA for treated samples against: (a): *S. aureus* and (b) *E. coli.*

Table 2
ANOVA results of antimicrobial activity against *E. coli* bacteria for treated samples.

Source	Sum of squares	df	Mean square	*F* value	*p*-Value prob > *F*	
Model	32536.82	9	3615.20	7.88	0.0017	Significant
A-TiO_2	23331.26	1	23331.26	50.88	<0.0001	
B-CA	7.84	1	7.84	0.017	0.8986	
C-SHP	0.94	1	0.94	0.00204	0.9648	
AB	1.58	1	1.58	0.0034	0.9544	
AC	1.82	1	1.82	0.0039	0.9511	
BC	1.58	1	1.58	0.0034	0.9544	
A^2	8605.62	1	8605.62	18.77	0.0015	
B^2	805.01	1	805.01	1.76	0.2147	
C^2	660.04	1	660.04	1.44	0.2579	
Residual	4585.57	10	458.56			
Lack of fit	4585.57	5	917.11			
Pure error	0.000	5	0.000			
Cor. total	37122.40	19				

R-squared: 0.8765, adjusted *R*-squared: 0.7653, CV% = 28.71%.

Table 3
ANOVA results of antimicrobial activity against *S. aureus* bacteria for treated samples.

Source	Sum of squares	df	Mean square	*F* value	*p*-Value prob > *F*	
Model	26910.71	9	2990.08	7.36	0.0022	Significant
A-TiO_2	18870.63	1	18870.63	46.42	<0.0001	
B-CA	103.99	1	103.99	0.26	0.6240	
C-SHP	92.57	1	92.57	0.23	0.6435	
AB	164.71	1	164.71	0.41	0.5387	
AC	149.48	1	149.48	0.37	0.5578	
BC	145.01	1	145.01	0.36	0.5636	
A^2	5717.32	1	5717.32	14.07	0.0038	
B^2	2246.98	1	2246.98	5.53	0.0406	
C^2	366.09	1	366.09	0.90	0.3650	
Residual	4064.90	10	406.49			
Lack of fit	4064.90	5	812.98			
Pure error	0.000	5	0.000			
Cor. total	30975.62	19				

R-squared: 0.8688, adjusted *R*-squared: 0.7507, CV% = 26.90%.

of microorganism cell wall resulting improved antimicrobial activity. These results show that antimicrobial property is not linear and it depends on the adsorbed Ag/TiO_2 on the wool as well as the concentration of cross-linking agent. In this condition, the wool surface is covered more and uniform in comparison with lower concentrations.

Analysis of variance (ANOVA) was used to analyze the data to obtain the interaction between the process independent variables and the responses. The results were then analyzed by ANOVA to assess the "goodness of fit". The Lack of fit describes the variation of data around the fitted model. If the model does not fit the data well, this will be significant (Tables 2 and 3).

It was observed that designed model for antimicrobial activity of treated samples against *E. coli* is statistically significant at *F* value of 7.88 and values of prob > *F* (<0.0017) (Table 2). Similarly, antimicrobial activity against *S. aureus* was significant at *F* value of 7.36 and values of prob > *F* (<0.0022) (Table 3). Additionally, Through *R*-squared coefficients the fit of the model can be evaluated. Regarding the antimicrobial activities against *E. coli* and *S. aureus*, the chosen models could not explain 12.35% and 13.12% of total variables, respectively. However, through adjusted *R*-squared it was confirmed that the model of antimicrobial activity against *E. coli* are more significance in comparison with that of *S. aureus*. In other words, their amounts are close to each other and their difference is not so obvious [41,42]. Finally, Low values of employed models CV% indicated a good precision and reliability of the experiments [41,42].

4. Conclusion

This research was conducted to introduce an effective nanocomposite to produce an ideal antimicrobial wool fabric. The Ag/TiO_2 nanocomposite was successfully applied on wool surface and stabilized through citric acid as a cross-linking agent. Its antimicrobial property against two well-known pathogenic bacteria *S. aureus* and *E. coli* was evaluated. Through XRD patterns, SEM images, and EDS spectrum the presence of Ag/TiO_2 nanocomposite on the surface of treated wool samples was confirmed. Increasing the amount of Ag/TiO_2 nanocomposite, citric acid, and sodium hypophosphite concentrations lead to enhance the antimicrobial activities. Moreover, RSM was employed to investigate the impact of independent variables on antimicrobial activity.

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