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In situ synthesis of silver nanoparticles on fiber matrix for preparing antibacterial paper

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ABSTRACT

In this study, the preparation of antibacterial paper was carried out using *in situ* synthesis of silver nanoparticles (AgNPs) on bleached cellulosic fiber matrix through chemical reduction of silver nitrate ($AgNO_3$) by two reducing agents of sodium borohydride (NaBH4) and formaldehyde (HCHO) under different molar ratio conditions. After synthesis, during the fabrication of hand-sheets, about 0.05% polyethyleneimine (PEI) was added to fiber suspension as retention aid to promote linking between fiber and nano-particles. The produced papers were then characterized by X-Ray Diffraction (XRD), UV-visible spectra analysis and scanning electron microscopy (SEM). In addition, the antimicrobial activity of AgNPs loaded papers was assessed. From the UV spectroscopy and antibacterial activity results, it was found that by increasing the molar ratio of HCHO:$AgNO_3$ and $NaBH_4$:$AgNO_3$, the maximum absorption, minimum wavelength, minimum particle size, maximum amount of produced nano-silvers and maximum percentage reduction of *E. coli* (Gram-negative) and *S. aureus* (Gram-positive) can be obtained. The optimum synthesized samples were gained at 0.05:0.001 ratio in which the formation of agglomerates was minimum. According to the electron microscopy images and X-ray diffraction results, nano-silver particles were formed in a spherical shape with an average crystallite size of 10-100 nm and located mainly on the surface of the fibers. Furthermore, it was demonstrated that the formation of unstable AgNPs agglomerates makes formaldehyde inferior to sodium borohydride as an efficient reductive agent. The contribution of PEI in retention and immobilization of AgNPs against washing and drainage during synthesis and hand-sheet making process was confirmed by SEM images. Hence, it could play an important role in controlling and reducing the migration of the silver particles to the environment. The present results show that *in situ* synthesis of AgNPs in fiber matrix can provide an appropriate approach in developing antibacterial paper-based products.

Keywords: *silver nanoparticles, in situ synthesis, paper, cellulosic fiber, antibacterial.*

1. INTRODUCTION

Nanotechnology has made great advances in different fields of science and technology. The novel properties of nanoparticles (NPs) have made them potential materials to be used in a variety of application areas, such as medicine, cosmetics, renewable energies, environmental remediation and biomedical devices [1-5].

The higher antibacterial activity of silver nanoparticles (AgNPs) relative to bulk silver metal makes them a good choice for producing antibacterial products [6-9]. Silver ions interact with the thiol groups of enzyme and proteins which play a vital role in bacterial survival [10-12]. It has been proved that cellulose fibers as biodegradable and renewable materials have great potential for functionalization with AgNPs to fabricate antimicrobial products [13, 14].

One of the superior properties of silver over that of other antimicrobial compounds is its higher toxicity to microorganisms even bacteria strains which exhibit resistance against antibiotics [15]. Although there are some arguments on the released silver ions into environment and subsequent ecotoxicity to aquatic organisms and about its toxicity against microorganisms [16, 17], most of the studies suggest its safe use with a maximum permitted dosage [18].

Several methods have been used in the past to prepare nano-structured silver particles, including chemical reduction of silver ions in aqueous solutions with or without surfactants, electrochemical reduction, heat evaporation, thermal decomposition in organic solvents, polyol process, chemical and photoreduction in reverse micelles and radiation chemical reduction [19-23]. Recently, biological methods have also emerged as viable options [8].

The main problem of all the mentioned methods is the tendency of the colloidal particles to clot into the solution [24]. Physical methods, due to their high temperature (higher than 1000 °C) requirements as well as expensive equipment and the complexity of the control of the synthesis condition, are less popular. Instead, chemical methods are more popular due to the ease of the control of reaction condition, simplicity and low cost of equipment and primary materials. Among the chemical methods, a chemical reduction method is more popular because of its simple equipment and operations [25, 26].

In this method, a reducing agent reduces silver salt into silver nanoparticles in an aqueous environment and the agglomeration of particles is avoided by using a proper stabilizer. The control of process conditions is important in order to obtain particles with identical sizes, shapes, and distributions. The most popular reducing agents in the preparation of silver colloids using chemical reduction are sodium citrate and sodium borohydride. This preparation is simple, but the great care must be exercised to make stable and reproducible colloids [27].

On the other hand, preparation of AgNPs-cellulose antibacterial products usually can be classified either as physical or chemical processes. Cellulosic materials in physical methods usually mix with nano-silver colloids and no chemical reactions

occur in the process. It is a simple and convenient operation and relatively high loading of silver can be achieved. However, due to relatively weak bonding of adsorption and consequently partial detachment of AgNPs from surfaces of cellulosic material during utilization, antibacterial efficacy of the product may be lost gradually. *In situ* chemical reduction in comparison with physical adsorption provides opportunity for AgNPs to grow slowly in cellulosic materials. In addition, this method exhibits better efficiency than physical adsorption. Small ionic radious of 0.1 nm enhances the probability of the silver ions penetration into the interior of the cellulosic fibers. After size increment, the embedment of AgNPs with cellulose networks prevents its loss [28]. Moreover, utilization of retention aids in the process like papermaking can help to keep them significantly inside the structure which was considered in this paper.

The antibacterial efficiency of the silver nano-particles together with the biodegradability of the fibers makes them practical for use as antibacterial paper. This kind of paper with antimicrobial properties could be found applications in health centers as wallpaper, writing paper, facemasks, tissue and as medical device packaging. Hence, the preparation of antibacterial paper was carried out in this study using *in situ* chemical reduction of silver nitrate ($AgNO_3$) by two different reactants of sodium borohydride ($NaBH_4$) and formaldehyde (HCHO) with different molar ratios in presence of cellulose fiber matrix. The antibacterial activities of the silver nano-particles loaded papers were then studied comparatively to show their action on bacteria.

2. EXPERIMENTAL SECTION

2.1. Materials.

Bleached softwood Kraft pulp was prepared from Tembec, Canada. Polyethyleneimine (PEI) with a molecular weight of 750,000 was supplied by Sigma-Aldrich. Sodium borohydride, ammonium hydroxide, and silver nitrate were all reagent grade and purchased from Merck and used as received. Formaldehyde as a reductive agent was prepared from Applichem, Germany. *Escherichia coli* (PTCC-1399) as a gram-negative model bacterium and *Staphylococcus aureus* (PTCC-1431) as a gram-positive model bacterium were provided by Microbiological Resources Centre, Department of Sciences and Food industry engineering, University of Tehran. The nutrient broth was purchased from Micro Media Company based in Hungary. Nutrient agar was obtained from the German company of Merck.

2.2. *In situ* synthesis of silver nanoparticles.

Silver nanoparticles were synthesized and precipitated on fibers using *in situ* method through chemical reduction of silver nitrate ($AgNO_3$) by two different reactants of sodium borohydride ($NaBH_4$) and formaldehyde (HCHO) with different molar ratios.

2.2.1. In situ synthesis of silver nanoparticles by $NaBH_4$. In this method to precipitate silver particles on the surface of fibers in a more homogenous way, the pulp was mixed with silver nitrate solution using a magnetic stirrer in a temperature of 25 ºC for 1 h. Then the suspension was left in darkness for 12 h. Afterwards, sodium borohydride solution (with certain concentrations of 0.001, 0.05 and 0.01 molar) as the reducing agent was gradually added to the suspension. The surrounding of suspension dish was covered by ice as the reaction is exothermic. While sodium borohydride was added to the suspension, the color of colorless silver nitrate solution gradually changed to light yellow, then to golden yellow and after that to silver. Finally, after 6 hours of dwell time, its color turned to brown.

After a 12-hour dwell time of fibers, in order to remove other materials produced during the reaction, the fibers were washed with double-distilled water. Generally, in different concentrations of sodium borohydride and silver nitrate, the process of changing color was observed indicating the formation of silver nanoparticles in the suspension.

Fig. 1 illustrates the schematic of loading of silver nanoparticles on fibers and equation (1) shows the reaction between silver nitrate and sodium borohydride [29].

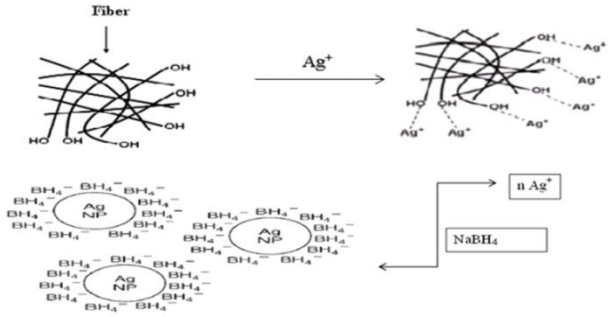

Fig. 1. Schematic representation of silver nitrate loading on fibers and chemical reduction by sodium borohydride .

$$AgNO_3 + NaBH_4 \rightarrow Ag + \frac{1}{2} H_2 + \frac{1}{2} B_2H_6 + NaNO_3 \qquad (1)$$

2.2.2. In situ synthesis of silver nanoparticles by HCHO. This method was carried out in two steps:

1- Impregnating fibers by silver nitrate as the supplier of silver ion with three concentrations of 0.001, 0.01 and 0.05 molar and by ammonium hydroxide as the supplier of hydroxide ion to provide alkaline conditions with three concentrations of 0.001, 0.01 and 0.05 M with the same ratio to silver nitrate.

2- Spraying formaldehyde (reducing agent) with different concentrations on fibers (0.001, 0.01, and 0.05 molar) at room temperature. About one hour after this step, based on the concentration of the used materials, the color of fibers was gradually changed to light yellow, golden yellow and in higher concentrations to orange.

If only silver nitrate and formaldehyde are used, the pH of reaction reduces and the reaction time for preparing silver particles increases. Hence, ammonium hydroxide was used to increase the pH of the reaction and to decrease the reaction time to less than one hour. After synthesis, in order to remove other produced materials, the fibers were washed with double-distilled water. Equation (2) depicts the reaction between silver nitrate and formaldehyde.

$$2\,AgNO_3 + 3NH_4OH + HCHO \rightarrow 2Ag + 2NH_4NO_3 + 2H_2O + HCOONH_4 \quad (2)$$

2.3. Making hand-sheets containing synthesized silver nanoparticles.

After synthesis and before fabricating hand-sheets, about 0.05% polyethyleneimine (PEI), a cationic polyelectrolyte with high charge density, was used as retention aid to provide a bridge linking between fiber and nano-particles. PEI provides sufficiently large positively charged patches on the surfaces to allow more effective electrostatic attraction between nanoparticles and fibers.

The pulp was washed in several steps in order to remove completely the free silver nano-particles. Then the hand-sheets were made using a Frank sheet former according to TAPPI T205 SP-06 standard at a basis weight of 60g/m^2. Three series of hand-sheets were prepared for each type of *in situ* loaded pulp.

2.4. Characterizations.

The effectiveness of the *in-situ* precipitation of silver nano-particles methods was evaluated on pulp and paper hand-sheets by various analyses and investigations.

2.4.1. X-Ray Diffraction (XRD). X-ray diffraction patterns of silver nanoparticles were obtained on an X–pert Philips, type: 3040/60 pw apparatus equipped with a transmission type goniometer, using nickel-filtered, CuKα radiation (λ = 1.5418Å) at 15kV. the goniometer was scanned stepwise from 20 to 80° in the 2θ range.

2.4.2. UV-visible spectroscopy. UV-Vis spectroscopy is a powerful tool for the characterization of colloidal particles and was used for monitoring the signature of silver nanoparticles. The optical absorption of silver nano-particle was measured using a UV-Vis spectrophotometer, UV-2450 (Shimadzu).

2.4.3. Scanning Electron Microscopy (SEM). The morphology of silver nano-particle loaded on paper samples were observed by using TESCAN-WEGA scanning electron microscope (SEM). The surfaces of the paper samples were coated with a thin conducting layer of gold.

2.4.4. Antibacterial activity. Antibacterial activities of silver nano-particle loaded papers were investigated against *Escherichia coli* as the model Gram-negative bacteria and *Staphylococcus aureus* as the model Gram-positive bacteria by following method [30, 31].

The colony forming count method:

Papers were cut into a disc shape with 1.5 cm diameter. Before inoculation of the bacteria, the pieces of the sample were sterilized by autoclaving at 120 °C for 15 min. The sample was divided into two groups; each group consists of eight pieces. The first group was seeded with 1 mL sterile nutrient broth as sterility control. The second group was seeded with fresh *E. coli* and *S. aureus* culture at a concentration of 10^7 and 10^9 colony forming units per milliliter (cfu/mL) respectively, then incubated in shaking incubator at 37 °C for 24 h. After incubation, 50 mL saline was added to each of groups and all tubes were then vortexed. After vortexing, about 50 µL of the suspension was drawn out of each tube by a 10-100 µL sampler and then distributed on a sterile plate. Next, 15-25 mL of the desired nutrient agar medium which was previously sterilized and its temperature reached 45 °C, was transported to the plate and completely mixed with the suspension using 8-like rotational movements. Finally, in order to count the number of colonies formed, the plates were put inside the incubator for 24 h at 37 °C. The same procedure was performed on pure unloaded paper samples. The percentage reduction in bacterial count was calculated as follows:

$$\text{Percentage reduction} = \frac{(\text{Viable count at 0 h} - \text{Viable count at 24 h})}{\text{Viable count at 0 h}} \times 100 \quad (3)$$

3. RESULTS SECTION

3.1. XRD of silver nanoparticles.

The X-ray diffraction (XRD) spectroscopy was used to examine the crystal structure of silver nanoparticles. The results confirmed the formation of silver nanoparticles using both synthesis methods with a variable molar ratio of reagents (Figs. 2 and 3). The XRD pattern of silver nanoparticles which formed on the surface of cellulose fibers shows the characteristic of four peaks of (111), (200), (220) and (311) planes of the metallic silver particles according to Ag XRD Ref. No. 00–004–0783 [32, 33]. The peaks showed that the main composition of nano-particles was silver and clearly no obvious peaks as impurities were found in the XRD patterns. Therefore, this gives a clear evidence of the presence of silver nanoparticles inside the paper structure.

The average particle size of silver nanoparticles was calculated using Debye–Scherrer equation (4):

$$n = \frac{K\lambda}{\beta cos\theta} \quad (4)$$

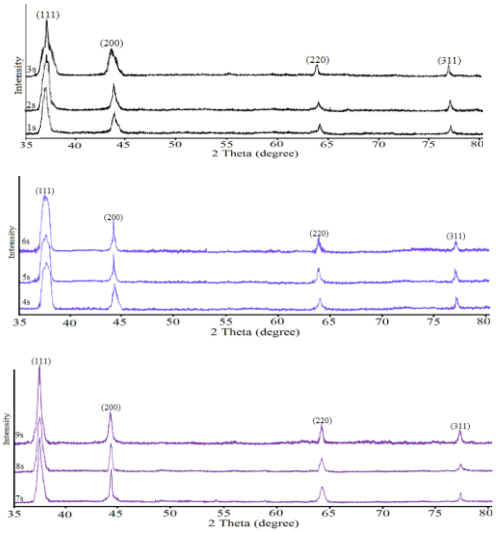

Fig. 2. The XRD patterns of silver nanoparticles *in situ* synthesized with different molar ratios of $NaBH_4$:$AgNO_3$. Sample 1 (0.001:0.001), sample 2 (0.01:0.001), sample 3 (0.05:0.001), sample 4 (0.001:0.01), sample 5 (0.001:0.01), sample 6 (0.001:0.01), sample 7 (0.001:0.05), sample 8 (0.01:0.05), sample 9 (0.05:0.05).

Where K is the Scherrer constant with value from 0.9 to 1 (shape factor), λ is the X-ray wavelength (1.5418 Å), β is the width of the XRD peak at half height and θ is the Bragg angle. From the Scherrer equation, the average crystallite size of the silver nanoparticles was found to be around 10-100 nm.

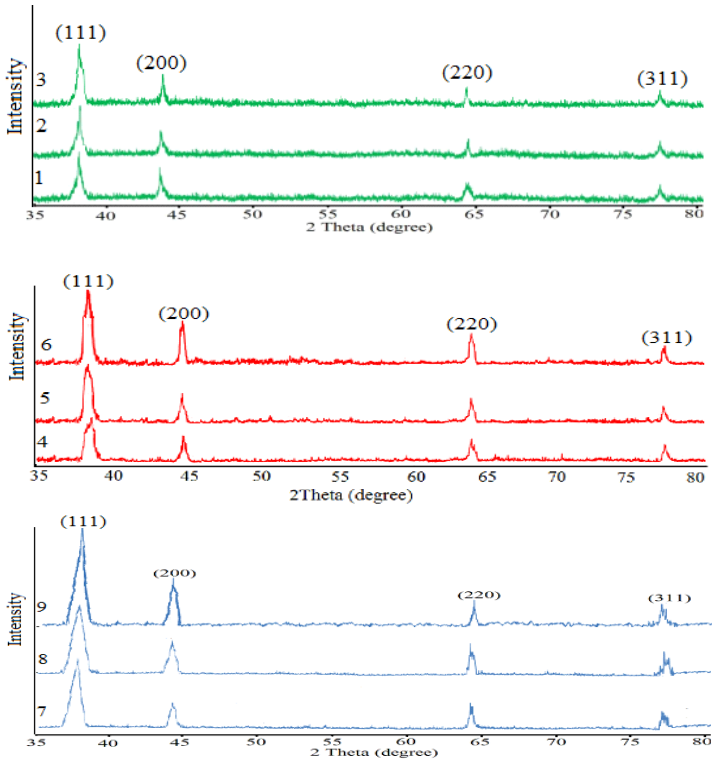

Fig. 3. The XRD patterns of silver nanoparticles *in situ* synthesized with different molar ratios of HCHO:$AgNO_3$. Sample 1 (0.001:0.001), sample 2 (0.01:0.001), sample 3 (0.05:0.001), sample 4 (0.001:0.01), sample 5 (0.01:0.01), sample 6 (0.05:0.01), sample 7 (0.001:0.05), sample 8 (0.01:0.05), sample 9 (0.05:0.05).

3.2. Absorption spectra of silver nanoparticles.

UV-Vis based methods utilize a shift in the maximum absorption as an indicator of particle size variations. These are effective for metallic particles such as gold and silver but are less suitable for non-metallic ones [34, 35].

Absorption spectra of silver nanoparticles on the surface of cellulose fibers for different molar ratios ($NaBH_4$:$AgNO_3$) and (HCHO: $AgNO_3$) are shown in Figs. 4 and 5. The observed red-shift and broadening of the absorption band are due to the increase in particle size and size distribution [31]. The reason can be justified by the high surface energy of nano-crystalline matters which is due to their large surface areas. In other words, the energy of electrons on the surface of the nano-crystalline varies from that of electrons inside the nano-crystalline. In addition, different energy balances are also considered on the surface. These energy differences are in the scale of the energy of UV waves. Therefore, when the ultraviolet (UV) wave is radiated to the desired material, the electrons which have a proper energy condition can absorb UV waves and be shifted to a higher energy level. Hence, the absorption of UV waves increases.

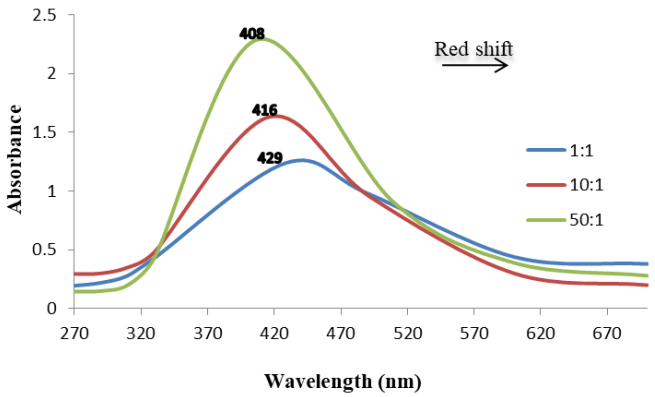

Fig. 4. Absorption spectra of AgNPs on the surface of cellulose fibers prepared by $NaBH_4$:$AgNO_3$ with molar ratios of 1:1(0.001:0.001), 10:1(0.01:0.001) and 50:1(0.05:0.001).

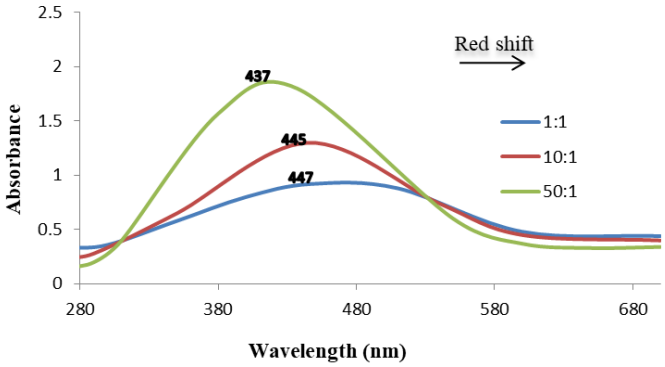

Fig. 5. Absorption spectra of AgNPs on the surface of cellulose fibers prepared by HCHO: $AgNO_3$ with molar ratios of 1:1(0.001:0.001), 10:1(0.01:0.001) and 50:1(0.05:0.001).

This implies that larger silver nanoparticles with a wider size distribution are probably formed when the $NaBH_4$/HCHO: $AgNO_3$ molar ratio decreases, which is due to the excess amount of silver ions aggregated together. In contrast, smaller silver nanoparticles with a narrow size distribution are formed when the $NaBH_4$/HCHO: $AgNO_3$ molar ratio increases. This is probably due to this fact that the amount of free electrons generated by $NaBH_4$/HCHO is high enough to prevent the aggregation of silver particles [31]. Therefore, the particle size and size distribution of silver nanoparticles can be controlled by adjusting the molar ratio of $NaBH_4$/HCHO: $AgNO_3$. Moreover, the $NaBH_4$/HCHO: $AgNO_3$ molar ratio influences the depth of silver nanoparticles inside the cellulose fibers. As it was shown in the figures 4 and 5 the absorption spectra of HCHO is broader and more red shifted than that of $NaBH_4$ indicating the larger particle size and wider size distribution of silver nano-particles in HCHO than in $NaBH_4$. It might be due to the agglomeration of AgNPs synthesized by HCHO.

3.3. Morphology of AgNPs precipitated on the surface of cellulose fibers.

According to XRD and UV spectroscopy results, it was observed that by increasing the concentration of sodium borohydride from 0.001 to 0.05 molar and consequently increasing the molar ratio of $NaBH_4$:$AgNO_3$, the maximum absorption, minimum wavelength and in turn minimum particle size can be obtained. The optimum synthesized samples were obtained at 0.05:0.001 ratios. In the samples with the high silver nitrate

concentration (0.05 molar), unstable agglomerates were probably formed but due to alower level, it was not detectable by XRD test. However, this condition was observable in electron microscopy images. According to the SEM images, nano-silver particles formed using *in situ* syntheses were usually in spherical shape and located on the surface of the fibers (Fig 6).

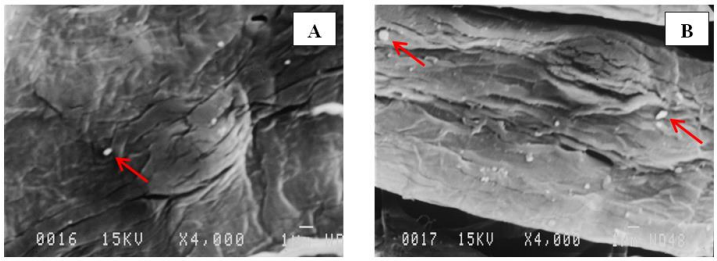

Fig. 6. SEM micrographs of AgNPs formed on the surface of cellulose fibers prepared from the $NaBH_4$:$AgNO_3$ molar ratio of 0.05:0.001 (a), 0.001:0.05 (b). The scale bar of (a) and (b) is 1µm (×4000).

In fact, these particles were formed regarding the reducing agent and the reaction condition. As the silver nitrate concentration increased and that of sodium borohydride decreased, more unstable agglomerates were formed which is in accordance with the UV spectroscopy results. However, the number and the size of these agglomerates were not high.

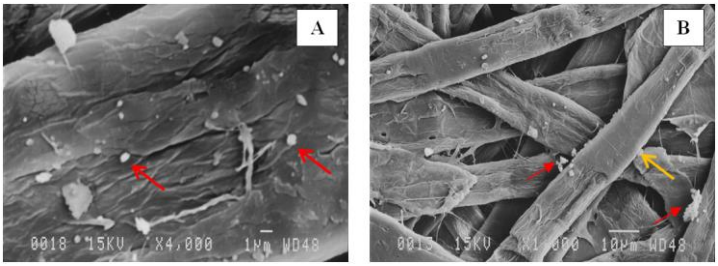

Fig. 7. SEM micrographs of AgNPs which formed on the surface of cellulose fibers prepared from the HCHO: $AgNO_3$ molar ratio of 0.05:0.001 (a), 0.001:0.05 (b). The scale bar of (a) is 1µm (×4000) and (b) is 10µm (×4000).

At a low and high concentration of silver nitrate, the formation of unstable agglomerates of silver nanoparticles was observed (Fig 7). These agglomerates were formed as agglomerations in some areas which represent that during silver nano-particles precipitation process by *in situ* synthesis, sodium borohydride performs better in comparison with formaldehyde as a reducing agent of silver nitrate.

The effect of retention aid, PEI, is evident on the surface of the cellulose fibers. This polymer had a contribution in keeping silver nano-particles during the washing process of the fibers which is done after synthesis and during paper preparation procedure. Hence, it could have an important role in reducing the migration of the silver particles and maintaining paper antibacterial efficacy.

3.4. Antibacterial activity.

It is worth to note that three general antibacterial mechanisms of silver nano-particles have been proposed which could damage the cell membrane and cause cell death in several investigations. In the first mechanism, nano-silver could be adsorbed or penetrate through the cell membrane. In the other mechanism silver ion released from the nano-silver could interact with the cell membrane and the intracellular component resulting in DNA damage. Nano-silver could form reactive oxygen species in the third mechanism [36].

3.4.1. Silver nano-particles loaded paper - sodium borohydride as a reducing agent. The results of antibacterial activity measurements of the prepared paper samples using in situ synthesis of AgNPs by silver nitrate and sodium borohydride reactants with different molar ratios are calculated in accordance with colony count method and provided in tables 1- 3.

The antibacterial activities of produced paper against *E. coli* as a Gram-negative bacterium and *S. aureus* a Gram-positive bacterium are reported here. The results demonstrate high antibacterial activity of silver nanoparticles against *E. coli* and *S. aureus*. As expected, as the molar ratio of $NaBH_4$:$AgNO_3$ increases, the number of AgNPs also increases and therefore the antibacterial activity and the percentage reduction of *E. coli* and *S. aureus* increase.

Table 1. Percentage reduction/increase in bacterial counts for papers containing AgNPs prepared from the $NaBH_4$:$AgNO_3$ molar ratio of (0.001:0.001), (0.01:0.001), (0.05:0.001).

$NaBH_4$: $AgNO_3$	***E. coli***				***S. aureus***			
	0.001: 0.001	0.01: 0.001	0.05: 0.001	Pure paper	0.001: 0.001	0.01: 0.001	0.05: 0.001	Pure paper
Reduction/ Increase	100% Red.	100% Red.	100% Red	32.5% Inc.	97.8% Red.	99% Red.	99.7% Red.	35.2% Inc.

As can be seen from table 1, growth and proliferation of bacteria are observed in the papers without nano-silvers (pure paper as control). The percentage increase is 32.52% and 35.20% for *E. coli* and *S. aureus*, respectively.

Table 2. Percentage reduction/increase in bacterial counts for papers containing AgNPs prepared from the $NaBH_4$:$AgNO_3$ molar ratio of (0.001:0.01), (0.01:0.01), (0.05:0.01).

$NaBH_4$: $AgNO_3$	***E. coli***			***S. aureus***		
	0.001:0.01	0.01:0.01	0.05:0.01	0.001:0.01	0.01:0.01	0.05:0.01
Reduction/Increase	100% Red.	100% Red.	100% Red.	100% Red.	100% Red.	100% Red.

Table 3. Percentage reduction/increase in bacterial counts for papers containing AgNPs prepared from the $NaBH_4$:$AgNO_3$ molar ratio of (0.001:0.05), (0.01:0.05), (0.05:0.05).

$NaBH_4$: $AgNO_3$	***E. coli***			***S. aureus***		
	0.001: 0.05	0.01: 0.05	0.05: 0.05	0.001: 0.05	0.01: 0.05	0.05: 0.05
Reduction/Increase	100% Red.	100% Red.	100% Red.	100% Red.	100% Red.	100% Red.

3.4.2. Silver nano-particles loaded paper - formaldehyde as a reducing agent. The results of antibacterial activity measurements of the prepared paper samples using in situ synthesis of AgNPs by silver nitrate and formaldehyde reactants with different molar ratios are also calculated in accordance with colony count method and provided in tables 4- 6.

Table 4. Percentage reduction/increase in bacterial counts for papers containing AgNPs prepared from the HCHO:$AgNO_3$ molar ratio of (0.001:0.001), (0.01:0.001), (0.05:0.001).

HCHO: $AgNO_3$	***E. coli***				***S. aureus***			
	0.001: 0.001	0.01: 0.001	0.05: 0.001	Pure paper	0.001: 0.001	0.01: 0.001	0.05: 0.001	Pure paper
Reduction/ Increase	81.4% Red.	88% Red.	92.1% Red.	37.3% Inc.	64.4 % Red.	69.6% Red.	80.6% Red.	39.9% Inc.

The results indicate the high antibacterial activity of silver nanoparticles against *E. coli* and *S. aureus*. As expected, as the

molar ratio of HCHO: $AgNO_3$ increases, the percentage reduction of *E. coli* and *S. aureus* also increases (Figs. 8 and 9). For example, for a molar ratio of 0.001:0.001 (HCHO:$AgNO_3$) the percentage reduction of *S. aureus* is 64.4% and that of *E. coli* is 81.4% which is more than *S. aureus* percentage reduction.

Table 5. Percentage reduction/increase in bacterial counts for papers containing AgNPs prepared from the HCHO:$AgNO_3$ molar ratio of (0.001:0.01), (0.01:0.01), (0.05:0.01) .

HCHO: $AgNO_3$	*E. coli*			*S. aureus*		
	0.001: 0.01	0.01: 0.01	0.05: 0.01	0.001: 0.01	0.01: 0.01	0.05: 0.01
Reduction/Increase	96.2% Red.	97.7% Red.	99.8% Red.	89.8% Red.	90.8% Red.	96.3% Red.

Table 6. Percentage reduction/increase in bacterial counts for papers containing AgNPs prepared from the HCHO:$AgNO_3$ molar ratio of (0.001:0.05), (0.01:0.05), (0.05:0.05) .

HCHO: $AgNO_3$	*E. coli*			*S. aureus*		
	0.001: 0.05	0.01: 0.05	0.05: 0.05	0.001: 0.05	0.01: 0.05	0.05: 0.05
Reduction/Increase	100% Red.	100% Red.	100% Red.	98.5% Red.	99.7% Red.	100% Red.

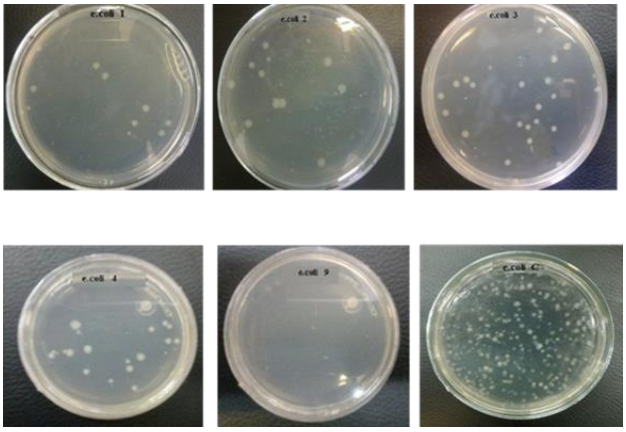

Fig. 8. Colony formation of *E. coli* (from the analysis of antibacterial papers). The code of samples is the same as figure 3.

The growth and proliferation of bacteria are also observed in the paper without nano-silver particles (pure paper). The percentage increase is 37.3% and 39.93% for *E. coli* and *S. aureus*, respectively (table 4).

According to the results of bacteria cultivation tables, silver nanoparticles have a significant role in the reduction of *E. coli* and *S. aureus*. The difference between the negative charge of microorganism and the positive charge of nano-particle functions as an electromagnetic absorber between bacterium and nano-particle and connects the nano-particle to the cell surface. Generally, silver nano-particles by affecting bacterial enzymes cause a significant stop in bacterial growth and are accumulated in vacuoles or cell wall. They also avoid cell division and damage cell envelope and bacterial contents. The size of bacterial cells increases. Cytoplasmic membrane, cytoplasmic contents and cell outer layer also show unusual structures [28].

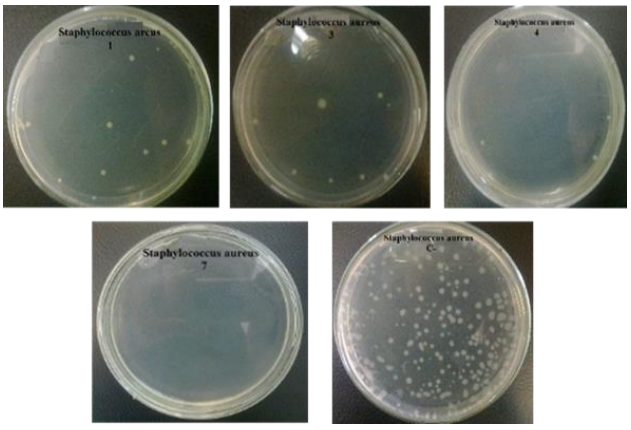

Fig. 9. Colony formation of *S. aureus* (from the analysis of antibacterial papers). The code of samples is the same as figure 3.

The antibacterial activity of silver nano-particles also largely depends on their size. Smaller nano-particles, in which surface-to-volume ratio is larger, have more flexibility. By increasing the surface of particles, the amount of adhesion to the cell surface increases and thus their permeability and germicidal ability increase. Such properties cause a higher germicidal ability of smaller nanoparticles compared to that of larger nanoparticles.

Therefore, as shown in the results, with increasing the ratio of materials in synthesis reaction and the amount of available nanoparticles, the size of particles decreases and thus the percentage reduction of both *E. coli* and *S. aureus* bacteria increase. In addition, the antibacterial activity of nano-particles against *S. aureus* bacteria is lower than that against *E. coli* which is due to the difference in cell wall between Gram-negative and Gram-positive bacteria. The Gram-negative cell membrane consists of lipopolysaccharide containing phosphate and pyrophosphate groups which render to the surface a density of negative charges superior to that observed for gram-positive ones, whereas Gram-positive bacteria (e.g. *S. aureus*) possess additional protection in the form of a dense peptidoglycan cell wall that provides effective protection against several biocides [12, 14, 31, 38, 39]. In general, it can be concluded that antibacterial materials by postponing the life cycle of the pathogen and reducing the growth speed, will affect its reproduction ability and finally destroy bacteria activity [40].

4. CONCLUSIONS

Regarding the results, it was observed that the antibacterial paper prepared by *in situ* synthesis of silver nanoparticles via silver nitrate reduction with sodium borohydride and formaldehyde, had a large effect on bacteria killing of both *E. coli* and *S. aureus* bacteria. However, this effect is more prominent for *E. coli* than for *S. aureus*. The electrostatic attraction between silver ions and hydroxyl groups of fibers surface causes silver ions to be placed on the fibers surface and in next step be converted to silver atom by sodium borohydride or formaldehyde reductive agent. Because of the high surface energy of these atoms, they connect to each other and produce silver nanoparticles. These nanoparticles, due to *in situ* synthesis and simultaneous loading, are placed mostly on fibers surface and in cell cavities.

PEI addition in fiber matrix caused to stabilize of the silver particles and increase their lasting time in paper structure. *In situ* synthesis method and simultaneous loading of silver, nanoparticles had the highest loading efficiency and wash stability. The

formation of silver nanoparticles was also demonstrated using absorption spectroscopy and X-ray diffraction.

Among the prepared antibacterial papers using *in situ* synthesis of AgNPs with the lowest concentrations of silver nitrate and sodium borohydride (0.001:0.001) and lowest concentrations of nitrate silver and formaldehyde (0.001:0.001), a reduction of 100% and 81.4% in *E. coli* (Gram negative) bacteria and a reduction of 97.8% and 64.4% in *S. aureus* (Gram-positive) bacteria were observed respectively.

The advantages of the prepared antibacterial papers using these low concentrations are their minimum economic costs, lowest silver toxicity and the best acceptable efficiency in terms of antibacterial properties. Hence, due to these advantages, they can be good candidates to be used in health centers as wallpaper, writing paper, facemasks, tissue and as medical device packaging.

These papers, in addition to ability in killing bacteria such as *E. coli* and *S. aureus* which enter from the environment, they are also able to kill the bacteria which are inside the paper structure. This feature is one of the most important advantages of the papers that allow the waste paper application to be extended in the packaging of cosmetics and foodstuffs and increase the portion of recycled fibers usage in this context.

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