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SILVER-CLAY NANOHYBRID AS A COATING FOR IMPROVEMENT OF THE ANTIBACTERIAL PROPERTIES OF PAPER

This comparative study was carried out to investigate the effect of nanosilver, silver-clay nanohybrid, and silver-milled clay nanohybrid coatings on the antibacterial characteristics of paper. Nanosilver (25 ppm) was used as a single- and double-layer coating. The results demonstrated that treatment with pure nanosilver was more efficient in the reduction of microbial growth. Also, milling of clay enabled better maintenance of silver nanoparticles, and led to a greater decrease in bacterial growth than in the case of the original silver-clay nanohybrid. Evaluation of the sustainability of antibacterial characteristics confirmed that, although pure nanosilver treatment achieved better performance in the first 15 minutes than nanohybrid samples, the performance of the nanohybrids improved with the passing of time. As expected, the treatments decreased the brightness of paper, while the opacity increased significantly; pure nanosilver treatment led to lower brightness than the others, and the opacity was higher in the case of the silver-clay nanohybrid than with the other treatments.

Keywords: paper coating; silver-clay nanohybrid; antibacterial property; *Escherichia coli*; *Staphylococcus aureus*

Introduction

The control of microorganisms as environmental contaminants has led to the development of antibacterial products [Imani et al. 2011]. Antimicrobial agents are used to prevent the growth of bacteria, viruses and fungi. Many chemicals and methods are used to accomplish this aim, and attempts have recently been made to replace them completely with effective chemicals and methods [Anderson et al. 2012]. In recent years, silver nanoparticles have played an important role in the development of nanotechnology [Rai et al. 2014]. Colloidal silver has been especially studied, due to its valuable properties in various

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scientific applications, such as biosensors, labels for cells and biomolecules, peptide probes, antimicrobial agents, and agents for wound therapy and cancer therapy [Chen and Chiang 2008; Thanh and Phong 2009; Ge et al. 2014]. The most important and exciting property of silver nanoparticles is their significant antimicrobial activity [Wasif and Laga 2009]. Silver nanoparticles have been successfully applied against various bacteria including *E. coli*, *Staphylococcus aureu*s, *Staphylococcus epidermis* and *Bacillus subtilis*, and also fungi such as *Aspergillus nigra*, *Candida albicans* and *Saccharomyces cerevisia* [Kim et al. 2007; Roe et al. 2008; Vertelov et al. 2008]. Among antimicrobial agents, silver has been used in the widest range of areas, because of its strong ability to extinguish pathogenic bacteria [Chen and Chiang 2008]. Moreover, inorganic nanoparticles have a high surface-to-volume ratio; they also possess unique physical and chemical properties [Bhat et al. 2008]. Therefore, the application of these nanoparticles to various fibers to create antimicrobial surfaces has been a subject of investigation. Many studies have been conducted on fiber/silver nanocomposites, among which the preparation of a suspension of fibers containing silver nanoparticles has proved very interesting. These investigations and products play an important role in such industries as textiles [Bhat et al. 2008; Chattopadhyay and Patel 2009; Girase et al. 2011]. By means of an active oxygen generating mechanism, silver particles act like an electrochemical cell, which produces oxygen ions via oxidation of oxygen atoms and OH^- ions through water hydrolysis; both of these are active bases and are also recognized as the most powerful antimicrobial agents [Sondi and Sondi 2004]. The presence of nanosilver in a paper matrix or composites is necessary to accomplish this chemical reaction. Also, in another mechanism, silver nanoparticles destabilize the potential of plasma membrane, reducing the ATP (adenosine triphosphate) level inside cells [Girase et al. 2011]. This function is performed via targeting of the plasma membrane of bacterial cells, leading to bacteria death [Sondi and Sondi 2004]. The application of silver nanoparticles as an antibacterial agent nonetheless has some major drawbacks, including stability, hydrophobic characteristics, and accumulation. The silver ions released from silver nanoparticles are oxidative, which entails a requirement for dissolved oxygen and protons. Oxidation of silver nanoparticles continues with the generation of peroxide. Peroxide has more powerful oxidation properties than oxygen, and under normal conditions it reacts with silver nanoparticles rather more quickly than oxygen [Girase et al. 2011; Barani 2014]. It seems that greater use of nanosilver can result in improvement of the antibacterial properties of silver, but with an increase in its cost.

Recently, the use of silver nanoparticles as an antibacterial agent has been considered in wet-end processes for some special types of paper, such as security and hygienic papers. However, adding this relatively expensive agent at the wet-end stage entails certain problems, including material loss during the dewatering process, random positioning of nanoparticles in the paper tissue, and the obtaining of relatively low antibacterial properties. The present research was based on a previous investigation of the antibacterial effects of adding Ag nanohybrids in pulp suspension in the wet-end section [Afra and Narchin 2017]. To solve these problems, in this research, antibacterial materials were applied as a coating layer using a coating process.

Considering the above-mentioned problems and reported research on coating systems, it appeared that the use of nanoclay in the production of silver-clay hybrids and the application of a silver-clay nanohybrid can result in improvement of the antibacterial properties of silver, as well as improving the surface and resistance properties of paper. This improvement is due to better stabilization of silver nanoparticles on the nanoclay particles, zero accumulation during reduction, and the increase in effective surface at the same charge. The aim of this research is to make a nanosilver/nanoclay composite with the ability to enhance the permanency of nanosilver, while also improving the surface and resistance properties of the coated paper.

Materials and methods

Materials

Full-bleached, 80 $g \cdot m^{-2}$, A4 paper sheets were used as paper samples. Sodium montmorillonite ($Al_2O_3 \cdot 4SiO_2 \cdot H_2O$) with a primary size of 44 μm was obtained from Sigma-Aldrich and employed for producing nanohybrid particles. Silver nitrate ($AgNO_3$) and sodium borohydride were obtained from Merck Co. Germany, and were also used for producing nanohybrid particles. Anionic starch ($C_6H_{10}O_5$) was supplied by Sigma-Aldrich, and used as binder.

Methods

Processing of silver nanoparticles

A reduction process was applied to produce silver nanoparticles. Accordingly, 200 mL $AgNO_3$ solution (with a concentration of 0.02 $mol \cdot L^{-1}$) was stirred at a rate of 1000 rpm at 60ºC for 8 h. The resulting solution was then centrifuged in two replicates at 25ºC at a rate of 5000 rpm for 15 minutes. Afterwards, the prepared mixture was dropped into 40 mL solution of sodium borohydride (at a concentration of 0.04 $mol \cdot L^{-1}$), which reduced Ag^+ ions to permanent metal nanoparticles. The container with the solution was then wrapped in foil and placed in a dark room for 24 hours. Finally, the silver nanoparticle solution was centrifuged at 25ºC at a rate of 5000 rpm for 30 min, and then washed twice.

Clay milling

To form discrete tactoid layers of nanoclay, natural clay was milled using a Narya MPM 2250/Amincomill apparatus, with 10 minutes of milling at a rate of 350 rpm followed by a 10-minute pause, for a total time of 20 h.

Nanohybrid processing

The process was initiated by adding 1 g of natural montmorillonite to 0.34 g of 0.02 $mol \cdot L^{-1}$ $AgNO_3$. The mixture was vigorously agitated manually for 30 min, and at 60ºC at a rate of 1000 rpm by a magnetic stirrer for 8 h. The resulting solution was thermally centrifuged at 25ºC at a rate of 5000 rpm for 15 min, twice. In the next phase, the resultant mixture (Ag^+ + montmorillonite) was added dropwise with stirring to a solution containing 0.03 g of 0.04 $mol \cdot L^{-1}$ sodium borohydride, and this was then wrapped in foil and placed in a dark room. The mixture was finally reduced from ionic to metallic form and was centrifuged again under the above-mentioned conditions for 30 minutes, when silver-clay nanohybrid was eventually obtained.

The procedure for preparing silver-milled clay nanohybrid was similar to the processing of simple silver-clay nanohybrid, except that clay that had been milled for 20 hours was used instead of natural montmorillonite.

Analysis of nanoparticles

Active light spectroscopy – dynamic light scattering (DLS) – was applied to measure the dimensions of the processed nanoparticles. The quantity of condensed silver nanoparticles on the clay layered surfaces was determined by inductively coupled plasma (ICP) spectroscopy.

Paper coating

After the processing of nanosilver and its hybrids with nanoclay, 2 g of anionic starch was prepared under continuous agitation in a water bath at 80-90ºC, and then applied to paper as a coating. After coating, air drying was employed to dry the paper. After 24 hours of drying of one side of the paper, it was placed on the coater again to coat the other side. These procedures were carried out for all three treatments: silver-clay and silver-milled clay nanohybrids, and nanosilver. Treatments and their specifications are shown in Table 1.

Measurement of paper properties

Physical tests including air resistance, burst strength, tear strength, opacity, and brightness were all conducted according to TAPPI standard test methods (T 460 om-96, T 403 om-97, T 414 om-98, T 425 om-96, and T 452 om-98).

Table 1. Coating treatments

Treatment code	Treatment description (coating of both sides of the paper)
C	control
Ag1	nanosilver based on 25 ppm for coating once
Ag2	nanosilver based on 25 ppm for coating twice
NHAgC1	silver–clay nanohybrid based on 25 ppm for coating once
NHAgC2	silver–clay nanohybrid based on 25 ppm for coating twice
NHAgMC1	silver–milled clay nanohybrid based on 25 ppm for coating once
NHAgMC2	silver–milled clay nanohybrid based on 25 ppm for coating twice

Biological testing was carried out in two different ways in this study. The first aimed to examine the antibacterial characteristics in various treatments. The second test was carried out to evaluate the permanency of antibacterial characteristics in various treatments, and also to assess the antibacterial activity in different treatments containing nanoparticles [Shrivastava et al. 2007; Sotiriou and Pratsinis 2010; Brooks et al. 2013]. The test was performed on *E. coli* and *Staphylococcus aureus* bacteria at the Provincial Health Center Laboratory of Gilan University of Medical Sciences (Gilan province, Iran).

FE-SEM, energy dispersive X-ray (EDX) and MAP images of coated papers were obtained to evaluate the percentage by weight, particle size and distribution of nanoparticles and silver-clay nanohybrids in the produced papers, and to assess the particle size and distribution of silver nanoparticles in nanoclays in the fabricated nanohybrids.

Results and discussion

Coating analysis

Figure 1 shows the results of DLS analyses. The diagram for the sample before ultrasonication (a) shows a sharp maximum peak of average-sized silver particles near 243 nm. The particle size distribution of the obtained silver particles ranged from 64 to 423 nm. Silver nanoparticles with smaller size are reported to have greater antibacterial activity than coarser ones [Mohtashemi et al. 2012]. It can be observed that after ultrasonication, the diagram (b) consists of two peaks; the larger peak contains approximately 92% of particles with sizes varying between 50 and 180 nm, while the particles belonging to the smaller peak lie in the range 10-25 nm. Since most of the particles were coarse before ultrasonication, it can be concluded that silver nanoparticles have a tendency to accumulate and agglomerate.

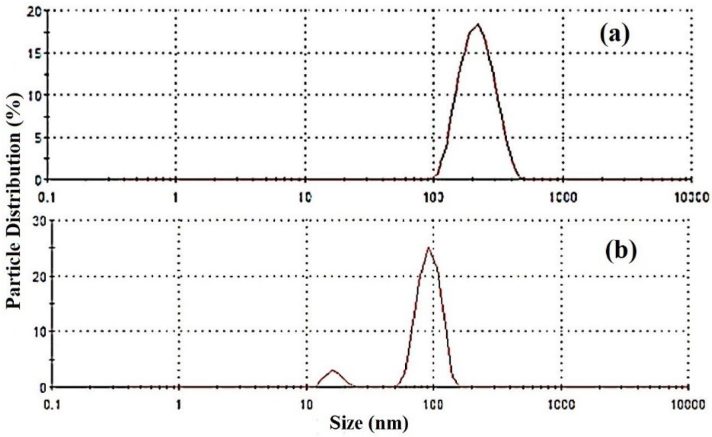


Fig. 1. DLS analysis of silver nanoparticles: (a) before ultrasonication and (b) after ultrasonication

In addition to atomic absorption spectroscopy (AAS), ICP spectroscopy was applied to confirm the desired concentration of nanosilver in the silver-clay and silver-milled clay nanohybrids (Table 2). The results revealed that the silver contents of the silver–clay nanohybrid and milled nanohybrid were respectively 5.3% and 10.85% of their dry weight.

Table 2. ICP results to calculate silver content in (a) silver-clay nanohybrid, and (b) silver-milled clay nanohybrid

(a)

Dosage (ppm): 10			V (ml): 25			Weight (g): 0.2508		
Nanosilver (%)	Calculated dosage	Int. (c/s)	RSD %	SD	Unit	Solution dosage	Wavelength (nm)	Element
5.30	13250	42886	0.4	0.36659	ppm	13.2924	328.068	Ag

(b)

Dosage (ppm): 25			V (ml): 20			Weight (g): 0.2507		
Nanosilver (%)	Calculated dosage	Int. (c/s)	RS D%	SD	Unit	Solution dosage	Wavelength (nm)	Element
10.85	54250	271131	1.3	1.1210	ppm	27.20095	328.068	Ag

The ICP results showed that the quantity of nanosilver absorbed onto silver--milled clay nanohybrid was twice that obtained for the silver-clay nanohybrid. This may be due to the milling process of the nanoclays: tactoid layers of clay were isolated and hence the exposed specific surface area of clay was increased, as the available negative charge of clay increased to create linkages with silver ions [Girase et al. 2011].

To study the particle size of solitary nanosilver and of nanosilver deposited on a clay surface and on milled clay in nanohybrids processed in a paper structure, and also the variation of clay particles during milling, FE-SEM imaging was applied. Figure 2 shows microscopic images of papers coated with nanosilver magnified by 5000×, 20000×, and 75000×. As can be seen, the silver nanoparticles have a uniform distribution on the paper surface. In the second and third rows of images, papers coated with silver-clay nanohybrid and silver-milled clay nanohybrid are shown. The silver particles are observed to lie in a size range around 50 nm.

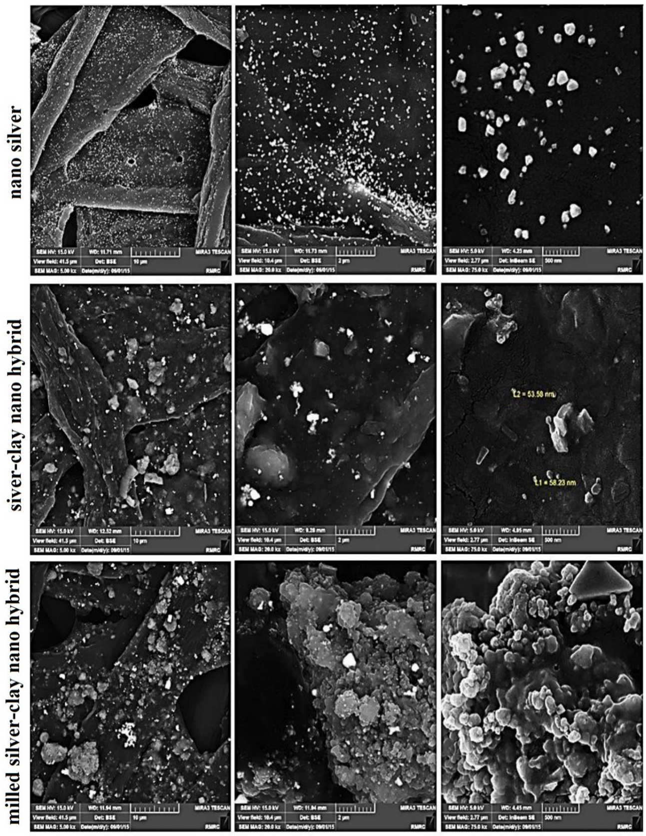


Fig. 2. FE-SEM images of papers coated with nanosilver, silver-clay nanohybrid, and silver-milled clay nanohybrid

Map images were taken at 5000× magnification from the surface of papers coated (once and twice) with silver–milled clay nanohybrid and nanosilver, based on 25 ppm of nanosilver. As the definition implies, map images show the elemental distribution on the surface [Wassilkowska et al. 2014].

The first row of images in Figure 3 shows paper coated with nanosilver and a map of silver nanoparticles, where the silver nanoparticles had a uniform distribution on the paper surface. The second and third rows of images show the surfaces of papers coated with silver-clay nanohybrid and silver-milled clay nanohybrid respectively, and also the distribution maps for these papers. Comparing the distribution maps of silver in nanohybrid silver coatings, it was found that the silver distribution in the hybrid treatments exhibits aggregation, where the formed aggregates vary depending on the use of unmilled or milled nanoclay.

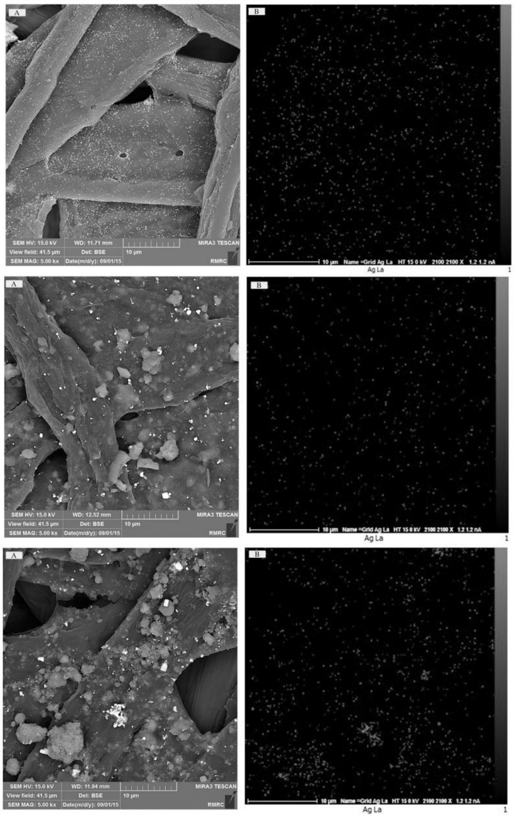
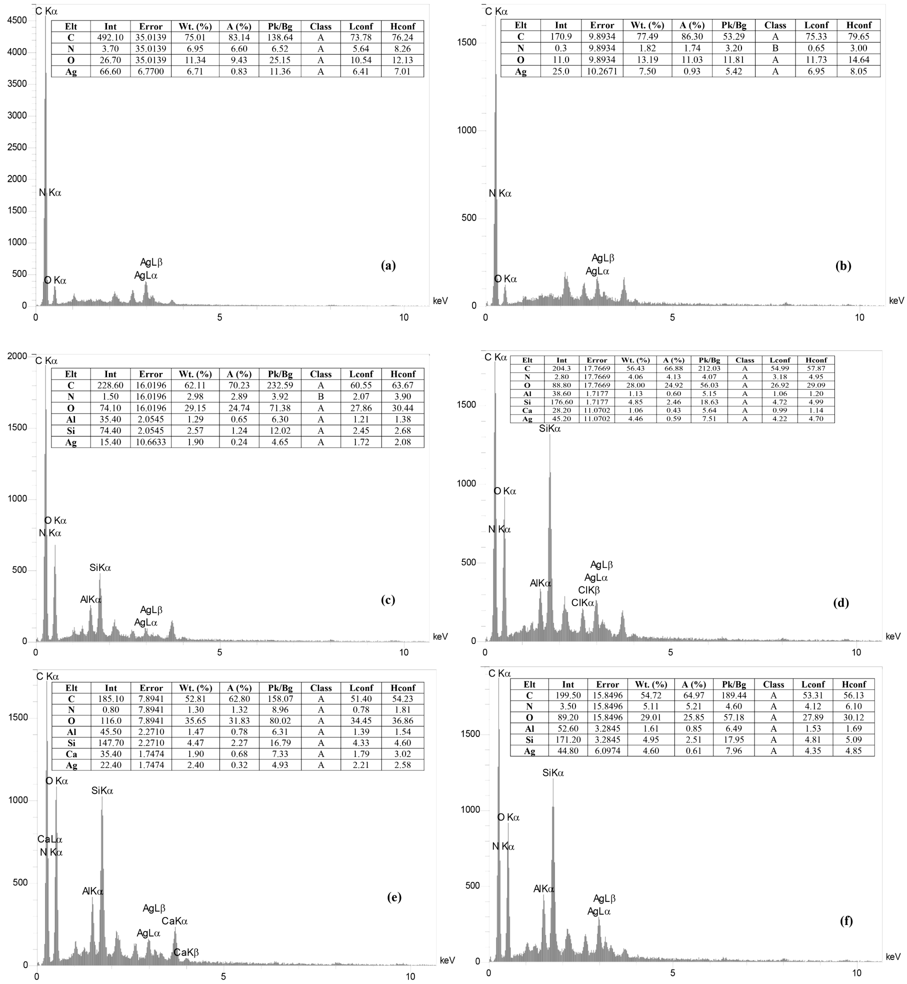


Fig. 3. FE-SEM map images. 1st row: (a) silver-coated paper, (b) map image of silver nanoparticle distribution; 2nd row: (a) paper coated with silver-clay nanohybrid, (b) map image of silver nanoparticle distribution; 3rd row: (a) paper coated with silver-milled clay nanohybrid, (b) map image of silver nanoparticles

Figure 4 illustrates the elemental composition of papers coated with silver nanoparticles. EDX analysis was used to specify the elemental composition of antibacterial coated paper [Wassilkowska et al. 2014]. As can be seen, silver accounts for 6.71 wt.% of the final paper surface in once-coated paper (Fig. 4a) and 7.50 wt.% in twice-coated paper (Fig. 4b). Thus, the quantity of nanosilver



(a)

Elt	Int	Error	Wt. (%)	A (%)	Pk/Bg	Class	Lconf	Hconf
C	492.10	35.0139	75.01	83.14	138.64	A	73.78	76.24
N	3.70	35.0139	6.95	6.60	6.52	A	5.64	8.26
O	26.70	35.0139	11.34	9.43	25.15	A	10.54	12.13
Ag	66.60	6.7700	6.71	0.83	11.36	A	6.41	7.01

(b)

Elt	Int	Error	Wt. (%)	A (%)	Pk/Bg	Class	Lconf	Hconf
C	170.9	9.8934	77.49	86.30	53.29	A	75.33	79.65
N	0.3	9.8934	1.82	1.74	3.20	B	0.65	3.00
O	11.0	9.8934	13.19	11.03	11.81	A	11.73	14.64
Ag	25.0	10.2671	7.50	0.93	5.42	A	6.95	8.05

(c)

Elt	Int	Error	Wt. (%)	A (%)	Pk/Bg	Class	Lconf	Hconf
C	228.60	16.0196	62.11	70.23	232.59	A	60.55	63.67
N	1.50	16.0196	2.98	2.89	3.92	B	2.07	3.90
O	74.10	16.0196	29.15	24.74	71.38	A	27.86	30.44
Al	35.40	2.0545	1.29	0.65	6.30	A	1.21	1.38
Si	74.40	2.0545	2.57	1.24	12.02	A	2.45	2.68
Ag	15.40	10.6633	1.90	0.24	4.65	A	1.72	2.08

(d)

Elt	Int	Error	Wt. (%)	A (%)	Pk/Bg	Class	Lconf	Hconf
C	204.3	17.7669	56.43	66.88	212.03	A	54.99	57.87
N	2.80	17.7669	4.06	4.13	4.07	A	3.18	4.95
O	88.80	17.7669	28.00	24.92	56.03	A	26.92	29.09
Al	38.60	1.7177	1.13	0.60	5.15	A	1.06	1.20
Si	176.60	1.7177	4.85	2.46	18.63	A	4.72	4.99
Ca	28.20	11.0702	1.06	0.43	5.64	A	0.99	1.14
Ag	45.20	11.0702	4.46	0.59	7.51	A	4.22	4.70

(e)

Elt	Int	Error	Wt. (%)	A (%)	Pk/Bg	Class	Lconf	Hconf
C	185.10	7.8941	52.81	62.80	158.07	A	51.40	54.23
N	0.80	7.8941	1.30	1.32	8.96	A	0.78	1.81
O	116.0	7.8941	35.65	31.83	80.02	A	34.45	36.86
Al	45.50	2.2710	1.47	0.78	6.31	A	1.39	1.54
Si	147.70	2.2710	4.47	2.27	16.79	A	4.33	4.60
Ca	35.40	1.7474	1.90	0.68	7.33	A	1.79	3.02
Ag	22.40	1.7474	2.40	0.32	4.93	A	2.21	2.58

(f)

Elt	Int	Error	Wt. (%)	A (%)	Pk/Bg	Class	Lconf	Hconf
C	199.50	15.8496	54.72	64.97	189.44	A	53.31	56.13
N	3.50	15.8496	5.11	5.21	4.60	A	4.12	6.10
O	89.20	15.8496	29.01	25.85	57.18	A	27.89	30.12
Al	52.60	3.2845	1.61	0.85	6.49	A	1.53	1.69
Si	171.20	3.2845	4.95	2.51	17.95	A	4.81	5.09
Ag	44.80	6.0974	4.60	0.61	7.96	A	4.35	4.85

Fig. 4. EDX analysis of (a) paper coated once with nanosilver, (b) paper coated twice with nanosilver, (c) paper coated once with silver-clay nanohybrid, (d) paper coated twice with silver-clay nanohybrid, (e) paper coated once with silver-milled clay nanohybrid, and (f) paper coated twice with by silver-milled clay nanohybrid

on the fiber surface is 11.70% greater in the case of twice-coated paper than for once-coated paper. For silver-clay nanohybrid treatments, the values obtained were 1.70 wt.% for once-coated paper (c) and 3.46 wt.% for twice-coated paper (d). It was also found that silicon accounts for 2.57 wt.% and 4.85 wt.% of the respective silver-clay nanohybrid-coated papers. The papers treated twice with silver-clay nanohybrid exhibited larger values (nearly twofold) for nanosilver and silicon than the singly treated papers, which confirmed the greater deposition of the hybrid coating on the paper. This significant difference between pure silver and silver-clay nanohybrid treatments is attributed to the lower concentration of nanosilver in the hybrids than in the suspension, which results in greater permeation and deposition of silver into paper surface pores. Finally, silver-milled clay nanohybrid-coated papers were subjected to elemental analysis. The measurements indicated that nanosilver accounted for 2.4 wt.% and 4.6 wt.% of the once-coated paper (e) and twice-coated paper (f) respectively, while silicon constituted 4.47 wt.% and 6.95 wt.% of the coated papers. As in the previous case, twice-coated paper had higher contents of nanosilver and silicon, which confirmed the larger quantities of hybrid coating in the paper. The higher levels of silver and silicon in the silver-milled clay nanohybrid-coated papers may be attributed to greater permeation of silver--milled clay nanohybrid into paper pores, due to its smaller dimensions in comparison with the nanohybrid with unmilled clay [Afra and Narchin 2017]. The silver contents of papers coated with nanosilver, silver-clay nanohybrid and milled clay nanohybrid were 6.71%, 1.70% and 2.40% respectively. This compares with reported values for retained silver of 0.49%, 2.76% and 1.23% when 25 ppm silver suspensions were used in the wet end of the papermaking process [Afra and Narchin 2017]. The reason for the greater deposition of nanosilver in coating than in wet-end application is the small dimension of the particles applied in coating, while in the wet-end case the retention of fine particles is a problematic issue [Mirshokrayi and Sadeghifar 2001].

Analysis of coated paper

The results revealed that air resistance was enhanced in all papers compared with the control sample (Fig. 5). Due to the presence of silver nanoparticles in the fine pores of paper, these particles increased air resistance to a certain extent. On the other hand, nanoclay and milled nanoclay enhanced the air resistance to a greater degree, because these particles could cover ultra-fine pores in the paper structure [Imani et al. 2011; Ottesen et al. 2017].

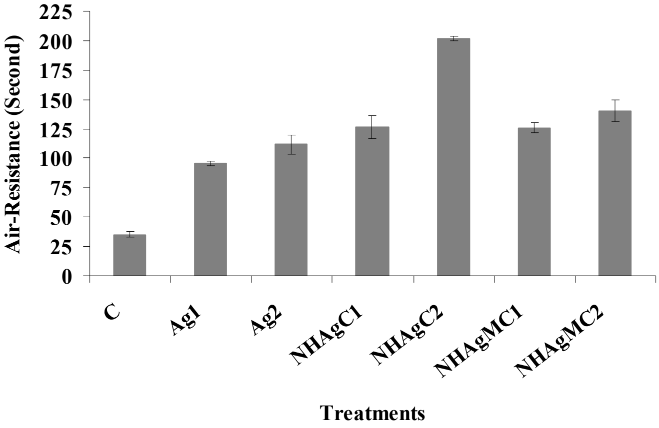


Fig. 5. Air permeability of various coating treatments

Mechanical properties, including burst and tear strengths, were not significantly changed by the various coating treatments (Fig. 6). This might be due to the fact that the relative bounded area was not affected during the coating process, and only weak coverage was created on the paper surface [Soares et al. 2012].

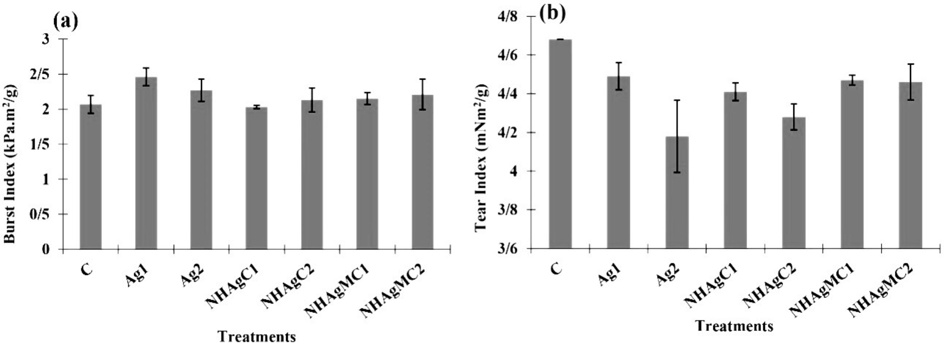


Fig. 6. Strength properties of different coatings: (a) burst and (b) tear

Figure 7 shows the effect of various treatments on paper brightness. As expected, the maximum brightness value was obtained for the control sample. Brightness decreased more in papers containing silver nanoparticles than in those containing nanohybrids. Silver nanoparticles were used in the form of a brown suspension, which absorbed light at 457 nm and decreased the brightness. Because nanoclay is more colorless than nanosilver, it will have a smaller effect on brightness; the content of silver in the hybrids is much lower than in the case of pure nanosilver, thus papers coated with pure nanosilver displayed a greater loss of brightness than those coated with nanohybrids [Afra and Narchin 2017]. Figure 7b shows the opacity of paper coated with nanosilver and nanohybrids compared with the control sample. Pure nanosilver increased the opacity via high absorption of visible light and a slight influence on the light scattering coefficient. However, papers treated with nanohybrids produced

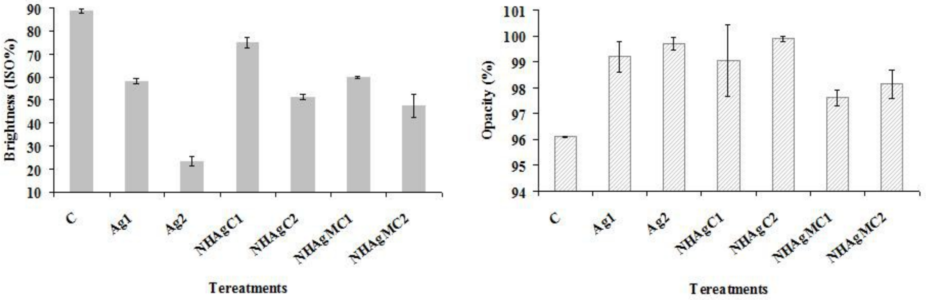


Fig. 7. Optical properties of coated paper

intense light scattering and average absorption of visible light, leading to opacity. The opacity of paper sheets is influenced by thickness, porosity, value and type of filler, degree of bleaching of fibers, coating, and the like [Zeinaly et al. 2016]. Coating increased the thickness of the paper, and so the opacity was increased.

Antibacterial activity

According to bacterial test results, pure nanosilver treatment showed better performance than the hybrid treatments in terms of decreasing bacterial growth (Figs. 8 and 9). Based on elemental measurements, the silver contents in papers coated once with nanosilver, silver-clay nanohybrid and silver-milled clay nanohybrids were 6.71%, 1.70% and 2.40% respectively. Thus, it is deduced that

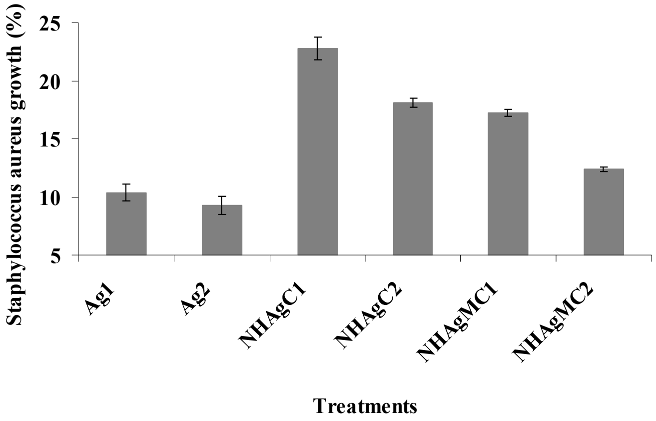


Fig. 8. Effect of various coating treatments on *Staphylococcus aureus* growth

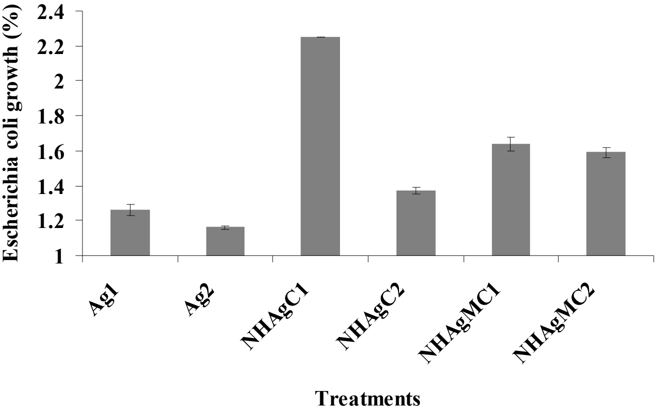


Fig. 9. Effect of various coating treatments on *Escherichia coli* growth

the higher silver contents in paper treated with pure nanosilver may be the reason for its more significant influence on bacterial growth [Vondruskova et al. 2010; Lafi and Al-Dulaimy 2011; Soares et al. 2012]. It was also found that antibacterial activity was enhanced when the coating procedure was performed for a second time, as this increased the quantity of silver deposited onto the paper.

Antibacterial tests revealed that the impact of nanosilver on *Escherichia coli* was more substantial than on *Staphylococcus aureus*. On the other hand, since small amounts of nanosilver can have a significant effect on *Escherichia coli* growth, the range of variation of growth retardation for this bacterium is small, and considerable reduction can be observed even for the minimal amount of nanosilver present in the silver-clay coating.

An antibacterial stability test was carried out to investigate the effectiveness of antibacterial activity for the treatments. Clearly, greater absorption of nanoparticles results in antibacterial activity of the paper. As shown in Figure 10, pure nanosilver coating was more efficient in reducing growth of *Staphylococcus aureus* for all mixing times (5, 30 and 45 minutes) due to higher silver deposition on the paper. When the time was increased to 30 and 45 minutes, however, the nanosilver coating displayed a constant effect on the growth of bacteria and was not able to improve it, which is attributed to the instability of silver particles and their rapid release from the paper surface [Girase et al. 2011]. Comparison of the two prepared nanohybrids showed that the silver-clay nanohybrid had a more durable influence than the milled nanohybrid. This is due to reclamation of silver particles in the layered structure of nanoclay tactoid layers, where slower and more regulated release of silver nanoparticles resulted in maintenance of their antibacterial effectiveness on paper [Girase et al. 2011].

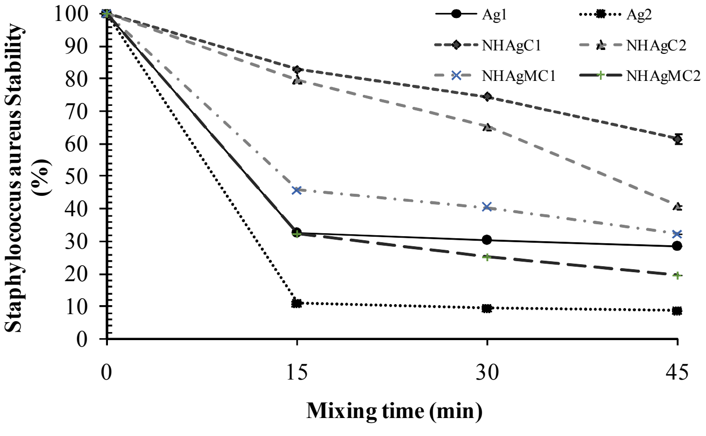


Fig. 10. Stability of antibacterial activity against *Staphylococcus aureus* over time

The stability of antibacterial activity against *Escherichia coli* was similar to that against *Staphylococcus aureus*, but since the effect of silver nanoparticles on *Escherichia coli* is greater than on *Staphylococcus aureus*, and accordingly the maximum reduction in microbial growth was reached at 15 minutes, the subsequent differences were small and insignificant (Fig. 11).

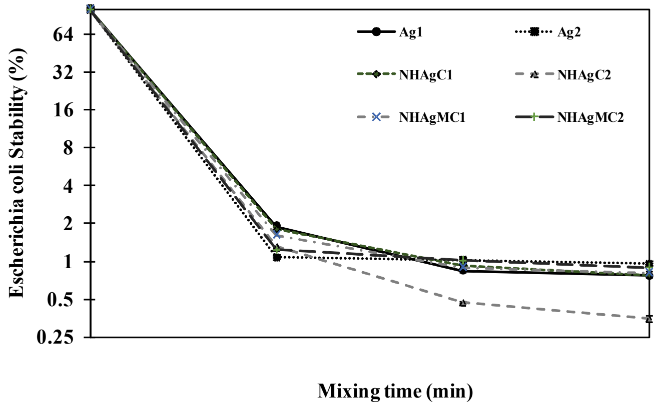


Fig. 11. Stability of antibacterial activity against *Escherichia coli* over time

Conclusions

The aim of this study was to prepare nanosilver, silver-clay nanohybrid, and silver-milled clay nanohybrid to be used as antibacterial coatings on paper. Elemental dispersive X-ray and FE-SEM imaging confirmed that the silver nanoparticle content in the pure silver coating was much higher than in the nanohybrid coatings. Antibacterial evaluations proved the higher efficiency of the pure nanosilver coating compared with the nanohybrids against both *Escherichia coli* and *Staphylococcus aureus* bacteria. The embedding of silver nanoparticles into nanoclay, or the formation of a silver-clay hybrid, improves the antibacterial characteristics of paper, particularly with the passing of time. Although the milling of nanoclay induced greater silver absorption than in the case of the silver-clay coating, the antibacterial properties of paper coated with silver-clay nanohybrid were maintained over time more effectively than when milled nanoclay was used, due to the regulated release of silver.

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