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ANTIBACTERIAL ACTIVITY AND THE HYDROPHOBICITY OF SILVER NANOPARTICLES LOADED FABRICS OF NYLON

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ABSTRACT

The objective of this research is to study antibacterial and hidrophobicity properties of Nylon 6.6 which coated nanoparticles - silver and hexadecyltrimethoxysilane (HDTMS). The nanoparticle - silver was prepared with chemical reduction method by using tri-sodium citrate as reducing agent and PVA as stabilizer. Nanoparticle - silver is deposited on fabrics of Nylon 6.6 as antibacterial agent and HDTMS is coated on those as hydrophobic agent. The fabrics of Nylon 6.6 are characterized by analyzing the functional groups using ATR-FTIR, hydrophobic properties by measuring contact angle, and antibacteria properties by measuring clear zone. The addition of HDTMS compound can decrease intensity of absorption bands of functional groups but increase hydrophobicity property of Nylon 6.6. Nylon 6.6 which coated nanoparticle silver and HDTMS has the highest antibacterial properties. The antibacterial properties of Nylon 6.6 before and after modification against *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* 32518 are different. All samples havedemonstrated a clear zone in inhibiting of growing bacteria *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 35218 at all the incubation time.

Keywords: antibacterial, contact angle, HDTMS, Nylon 6.6 fabric, silver nanoparticles.

INTRODUCTION

Polymeric materials especially textiles have extraordinary range of properties so that become a very important part of our day - to day life. Some applications of textiles include healthcare products and biomedical material made of various forms of textiles. For example, polypropylene is being widely used as support to repair hernias in a surgical procedure [1]. The antibacterial polymers can be developed by loading silver nanoparticles on textile materials, for example on cotton cloth [2], wool [3], polyester [4], polyamide [5] and silk [6] as biomedical material. The prevention of adsorption and growth of microorganisms on textile surfaces is prerequisite for the biomaterials to prevent infections. However, synthetic textiles themselves do not have antibacterial properties. Therefore, antibacterial agents have been used. The chemical agents have been used for inhibiting microorganisms growth, such as silver, copper, and other metal ions. Recently, nanosized silver nanoparticles (AgNPs) have been reported to exhibit antibacterial properties against representative pathogens of bacteria [7]. The silver nanoparticles showed synergistic effect with levofloxacin antibiotic, the antibacterial activity increased by 1.16 - 1.32 fold [8]. Silver nanoparticles can be synthesized via chemical reduction method [9] and green synthesis method [8]. $AgNO_3$ solution is used as the base material and trisodium citrate is used as a reducing agent. In addition, the antibacterial properties of silver nanoparticles is influenced by the particle size, the smaller the size of the silver nanoparticles were more efficient in the antibacterial activity tests.

Silver nanoparticles can be coated onto polyurethane foams in diverse forms as an antibacterial water filter and can be washed several times without any loss of nanoparticles [10]. The cotton fabrics incorporated with silver nanoparticles showed no bacterial growth, so that this material can be used to turn sterile fabrics [11]. Superhydrophobic conductive cotton textiles with antibacterial activity were synthesized successfully by in situ coating textiles with AgNPs followed by hydrophobization [12]. This method to multifunctionalizing conventional textiles with combined properties of superhydrophobicity, antibacterial, conductivity, etc.

One effort should be made to develop a self-cleaning textile products and antibacterial, by developing a hydrophobic textile material, followed by application of nanoparticles effectively and selectively. The nanoparticles can kill many types of microbes in a broad spectrum, but is not toxic to pathogenic microbes. Textile materials with hydrophobic properties can be obtained by adding silane compound. A modification technique to produce the hydrophobic textile materials is by addition of HDTMS molecules with sufficient hydrocarbon chain length and low surface energy [13].In this research, silver nanoparticles haveprepared by applying the bottom-up approach using reduction process. Then the textile material is coated with silver nanoparticles. Furthermore, the textile material that has been coated by silver nanoparticles, is modified by adding HDTMS to produce hydrophobic textile.

MATERIALS AND METHODS

Chemicals and instrumentation

Nylon 6.6 was purchased from the fabric store in Yogyakarta. Silver nitrate, trisodium citrate, polyvinyl alcohol (PVA), ethanol, acetone, and

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hexadecyltrimethoxysilane (HDTMS) were purchasedfrom Merck as commercial products and used as they were without any further purification. Nutrient Agar (NA) and Nutrient Broth (NB) were purchased from Oxoid. Nitrogen gas was purchased from PT Samator, Yogyakarta. *Staphylococcus aureus* ATCC 25923 and *Eschericia coli* 32518 were obtained from collection of Faculty of Medicine, Universitas Gadjah Mada, Indonesia.

The tools used include autoclave, oven, micropipette, reflux tools, laminar flow, incubator, caliper, camera, and drygalski. Some instruments operated for analysis were UV-Vis spectrophotometer (Shimadzu UV-2400PC series, Japan) and Fourier Transform Infrared-Attenuated Total Reflactance spectrophotometer (Perkin Elmer FTIR-ATR, Japan).

Procedure

Synthesis of nanoparticle - silver

Preparation of nanoparticle - silver was performed by preparing $1x10^{-3}$ M silver nitrate solution, 10% trisodium citrate solution, and 0.2% PVA solution. PVA solution and silver nitrate solution were added into three neck flask then refluxed until temperature of 90^0C [14, 15]. Trisodium citrate solution was added dropwise. When the reflux process was ongoing, gas N_2 was flowing until reflux process finished. Heating and flowing of N_2 gas were stopped if already transformed solution into a yellow, but stirring was stil done until room temperature reached. Colloidal of silver nanoparticles was characterized using UV-Visspectrophotometer.

Application of silver nanoparticles on Nylon 6.6 cloth (Nylon 6.6 - nanoAg)

Nylon 6.6 is cut to the size of 7cm x 7cm. The Nylon 6.6 fabrics that have been washed, then dried using hairdryer. The application of silver nanoparticles on Nylon 6,6 was done by immersion method. Nylon 6,6 cloth is immersed in colloidal of nanoparticle - silver then twisted around using a shaker with a speed of 155 rpm for 24 hours. Samples that have been soaked then dried.

Modification of surface of Nylon 6.6cloth with HDTMS (Nylon 6.6 - HDTMS)

HDTMS is dissolved in ethanol and then the Nylon and the Nylon -nanoAg are dipped into the HDTMS solution. The reacting process between HDTMS and ethanol solution was carried out at room temperature for 60 minutes. Nylon and Nylon-nanoAg which dipped in HDTMS solution are dried at room temperature. Nylon and Nylon-nanoAg that have been through the process of modification with HDTMS called the Nylon – HDTMS and Nylon - nanoAg-HDTMS. Nylon before and after modification are analyzed by using FTIR-ATR spectrophotometer, antibacterial activity test, and contact angle test.

The sample which prepared in this study are Nylon cloth (N0), Nylon cloth – nano Ag (N1), Nylon cloth - HDTMS (N2), and Nylon cloth -nanoAg - HDTMS (N3).

Characterization

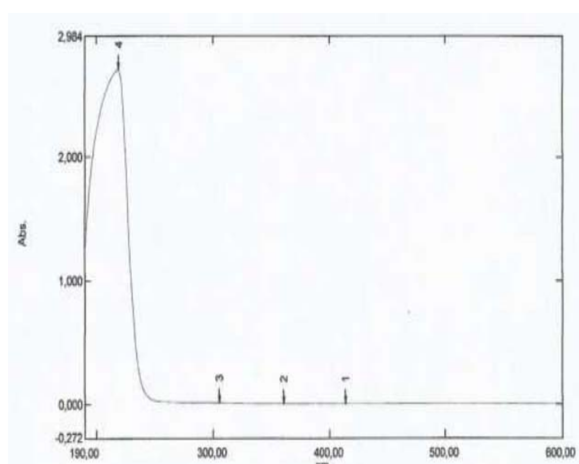
The characteristic of nanoparticle - silver was performed using UV-Vis spectrophotometer. An absorbance of silver nitrate solution $1x10^{-3}$ M and silver nanoparticle, are measured using a reference solution of distilled water. The functional groups of Nylon fiber samples before modification, after being coated silver nanoparticles, after being coated with HDTMS and after being coated with silver nanoparticles and HDTMS is analyzed by using Fourier Transform Infrared-Attenuated Total Reflactance spectrophotometer.

The properties anti-dirty (hydrophobic) of the samples were determined by measuring the water contact angle (θ) between the fluid and the sample surface [1]. The images processed using software to determine the contact angle between the liquid surface of the sample.

Antibacterial property was performed by preparing bacterial growth media such as Nutrient Agar and Nutrient Broth by dissolving 14 grams of NA in 500 mL of distilled water and 2 grams of NB in 250 mL of distilled water. All the tools and media for growing bacteria were sterilized in autoclave. Rejuvenation of *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 32518 was performed on an agar medium NA and incubated for 24 hours at room temperature. *Staphylococcus aureus* 25923 and *Escherichia coli* ATCC 32518 which has been rejuvenated for 24 hours and then inoculated into a liquid medium NB in the culture bottles and incubated for 24 hours at a temperature of 37^0C. Meanwhile, NA poured into each petri of approximately 10 mL and wait about 24 hours anyway. Each sample is cut with a diameter of 0.5 cm inserted into the petri dish and allowed in the incubator for 24 hours, then observed a clear zone.

RESULTS AND DISCUSSIONS

Figure-1 shows the spectra UV-Vis of the silver nitrate solution and nanoparticles - silver.Peak at wavelength region 429 nm indicates that it has formed nanoparticles Ag^0. This is consistent with research Barud *et al*. [16], Saputra *et al*. [14], and Ibrahim [8].



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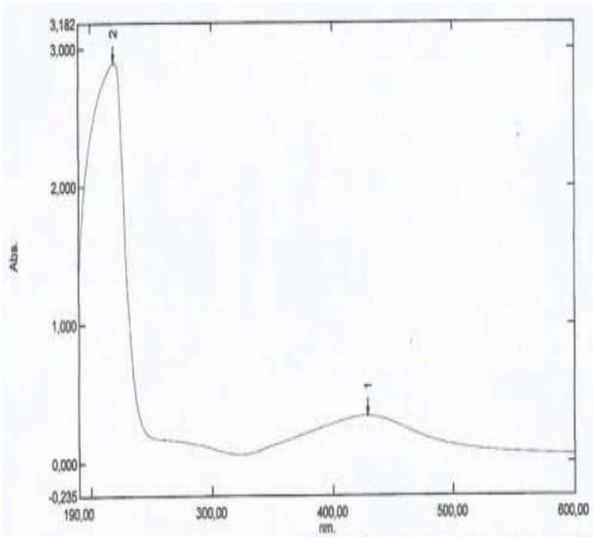

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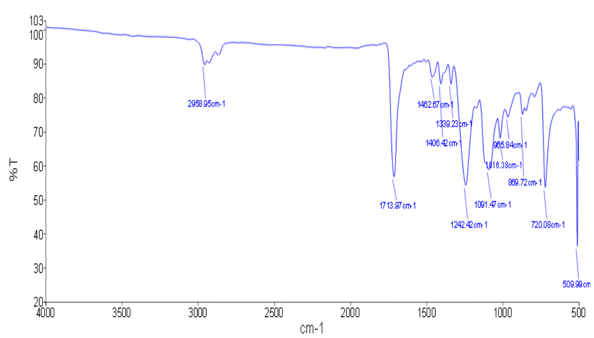
Figure-1. The spectra Uv-Vis of (a) the silver nitrate solution, (b) silver nanoparticle.

From the analysis of the functional groups (Table-1) shows that all samples of Nylon 6.6 with and without modifications have functional groups alkyl, C = O, N - H deformation, amides, C - N, C - C stretching, N - H wagging, and C-H rocking. Based on Figure 2 can be seen that there was no significant difference among functional groups of Nylon 6.6 (N0), Nylon 6.6 - nanoAg - silver (N1), Nylon 6.6 - HDTMS (N2), and Nylon 6.6-nanoAg - HDTMS (N3). But the intensity of functional group of the Nylon 6.6 - HDTMS and Nylon 6.6 - nanoAg - HDTMS are lower in comparison Nylon 6.6 (N0) and Nylon 6.6- nanoAg (N1).



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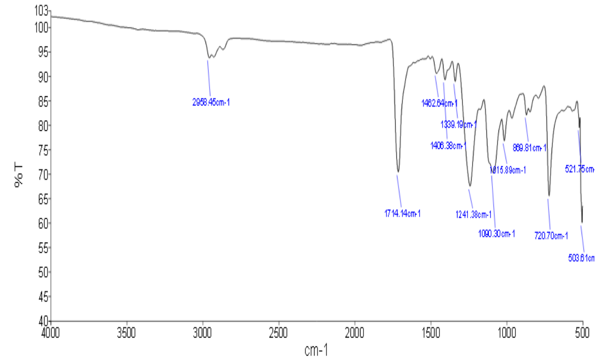

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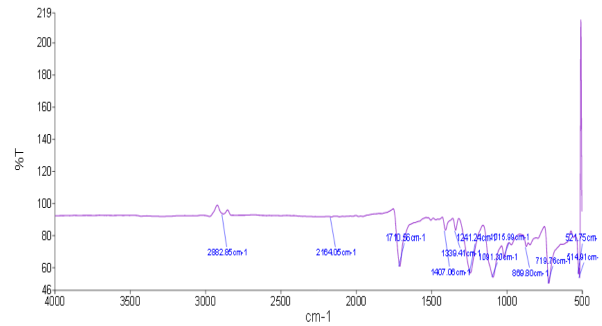

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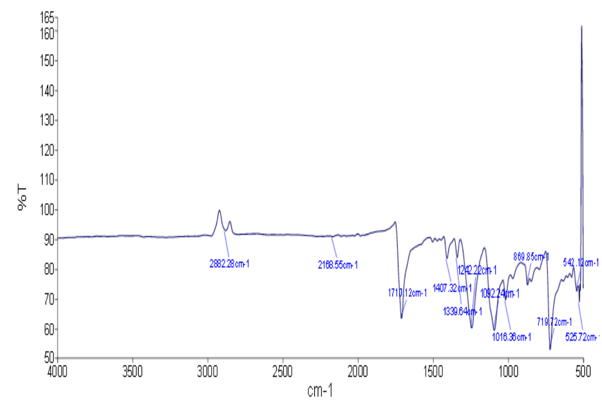

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Figure-2. The FTIR-ATR spectra of (a) N0, (b) N1, (c) N2, and (d) N3.

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Table-1. Functional groups of Nylon 6.6 before and after modification.

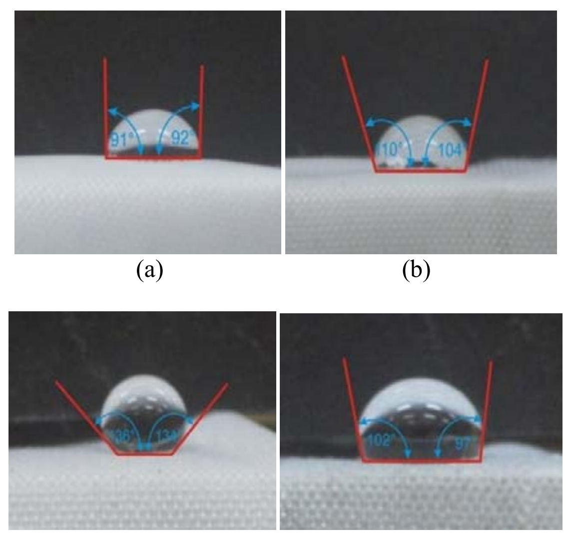
Interpretation of functional groups	Wave number (cm^{-1})			
	N0	**N1**	**N2**	**N3**
C=O stretching	1713.97	1714.14	1710.56	1710.12
Alkyl	2958.95	2958.45	2882.85	2882.28
C-N	1242.42	1241.38	1241.24	1242.22
N-H deformation	1462.67	1462.64	1407.06	1407.32
Amide	1339.23	1339.19	1339.41	1339.64
C-C stretching	1016.38	1015.89	1015.99	1016.36
N-H wagging	869.72	869.81	869.8	869.85
CH_2 rocking	720.08	720.7	719.76	719.72

This can occur because HDTMS compound coated fabric of Nylon 6.6 (N2) as a whole so that the infrared radiation was blocked by the HDTMS compound. Likewise with intensity of Nylon 6.6 - nanoAg- HDTMS decreased. In addition, infrared spectroscopy is actually very good for the determination of functional groups of organic compounds, while the silane compound is an inorganic. There should be the use of methods to determine the silane groups in fabric by using other methods. One of them using the XPS method so that it can more clearly study the energy from the silane bonding such as in the cotton cloth [17]. However, the addition of nanoparticles - silver toward fabrics of Nylon 6.6 did not change intensity of functional group of Nylon 6.6, because of the size of the nanoparticles is not so affect the structure of the fabric of Nylon 6.6. Nanoparticles - silver spread to the fibers and does not cover the surface of fabrics so that the infrared radiation can be transmitted properly.

Table-2 and Figure-3 provide information that the highest contact angle is on a sample of Nylon fabric which coated HDTMS without silver nanoparticles i.e. 135^0. HDTMS can bind to the -NH of Nylon cloth to form Si-OH and provides hydrophobic properties. Additionally, HDTMS has an alkoxide group and a long alkyl chain that has the ability to hold water well [12]. Silane compounds have a characteristic that can provide very low surface free energy on the surface of the fabric treated with the compound [18]. The modification of the material with a HDTMS will produce a rough surface that will reduce the surface free energy and can cause material properties that more hydrophobic[17].

Table-2. Contact angle of Nylon before and after modification.

Contact angle	**Nylon**	**Nylon-nanoAg**	**Nylon-HDTMS**	**Nylon -nanoAg-HDTMS**
Right angle	91^0	110^0	136^0	102^0
Left angle	92^0	104^0	134^0	97^0
Contact angle (average)	91.5^0	107^0	135^0	99.5^0



(a) (b) (c) (d)

Figure-3. The contact angles of (a) N0, (b) N1, (c) N2, and (d) N3.

The contact angle decreased with the increasing of alkyl chain of silane. Alkyl with carbon 16 (C16) can produce the hydrophobic fabric. Hydrophobic properties due to very rough surface which formed by a layer of particulate matter [18]. The binding between HDTMS compounds with Nylon fabrics can produce hydrophobic Nylon fabrics. The difference between the contact angle of a pure Nylon cloth with a Nylon cloth - HDTMS is significantly different. However, the addition of silver nanoparticles decreases the hydrophobic properties of the fabric. It is reported in previous studies that the addition of these nanoAg particles to hydrophobic materials, such as composite resins, causes an increase in surface energy and a reduction of the contact angle, which is in accordance with the results of the present study. The addition of 0.5% to the composites caused a decrease in the contact angle of water [19].

Antibacterial activity of Nylon cloth against *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 32518

Antibacterial activity test is done by using *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 35218. The parameter used in this analysis is the diameter of the clear zone that appears at around the sample. The clear zone around the sample formed by the antibacterial activities of the sample so that bacteria does not grow in this area. The wider clear zone diameter indicates a more effective inhibition against bacteria of the tested sample.

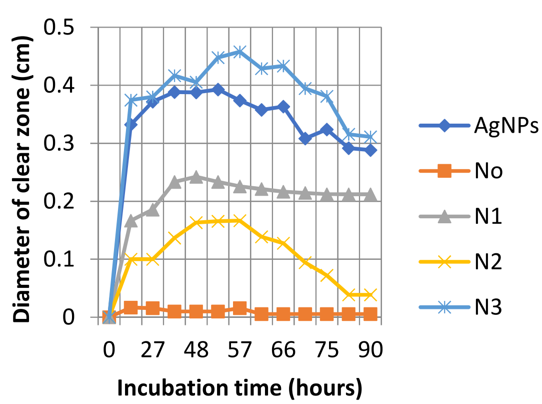
Table-3 shows that all test samples have shown a clear zone in inhibiting of growing bacteria *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 35218 at all the incubation time. Sample N_3 shows the highest antibacterial activity against bacteria *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 35218. Nevertheless, the antibacterial activity of the lowest is the sample of N0. Overall, increased incubation time can cause increasing of inhibition zone of Nylon untill 48 hours incubation except N0 and N2 decreased theirs inhibition zone diameter.

Table-3. Antibacterial activity of Nylon 6.,6 cloth against *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 35218.

Incubation time (Hours)	**Average diameter of the clear zone** (mm)							
	Staphylococcus aureus				***Escherichia coli***			
	N0	N1	N2	N3	N0	N1	N2	N3
24	0.2	1.7	1.0	3.7	0.8	3.6	2.5	2.8
48	0.1	2.4	1.6	4.1	0.9	3.9	1.9	3.5
60	0.1	2.2	1.4	4.3	0.8	3.3	1.2	4.0
72	0.1	2.1	0.9	3.9	0.7	3.0	1.0	4.1
90	0.1	2.1	0.4	3.1	0.6	2.9	0.7	3.4

The diameter of inhibition zone on all samples tend to decrease after the incubation time of 48 hours except N2 and N3samples. Of the four samples tested, the N3 samples (Nylon -nanoAg - HDTMS) showed the highest clear zone. Figure 4 shows the sequence of antibacterial activity of Nylon 6.6 began lowest to highest are as follows: N0<N2<N1<N3. The lowest antibacterial activity against *Staphylococcus aureus* is sample of N0 and the highest antibacterial activity in the samples of N3. For samples of N0 showed very low inhibition zone. This happens because N0 samples that do not have antibacterial properties. Diameter of zone of inhibition produced by the sample of N2 is not so great because the N2 samples don't have antibacterial activities. However, the adding of HDTMS toward N2 cause N2 samples have hydrophobic properties. The N2 samples show diameter of the inhibition zone slight. On the N1 samples reached the top of the highest inhibition zone diameters at 48th hours and after that the diameter of inhibition zone unchanged. The highest peak of the diameter of inhibition zone was samples of N3 at the 57th hour.

The nanoparticles - silver (AgNPs) have a large surface area so as to facilitate their contact with microorganisms. Silver nanoparticles kill bacteria via the process of diffusion [20]. Research on antibacterial silver nanoparticles are also performed by Patel *et al*. [21] which states that the antibacterial properties of nanoparticles associated with its small size, large surface area that makes the interaction with the higher microbial membrane. Oxygen of -OH in cotton cloth bound to the silver will bind to sulfhydryl (-S-H) on the cell membrane to form a bond R-S-S-R and produce S-Ag clusters that cause lethal inhibition of cell respiration. Cluster S-Agis very stable on the cell surface of bacteria because bacteria have sulfhydryl compounds that are not owned by mammals. The silver nanoparticles are not toxic in animals and humans.



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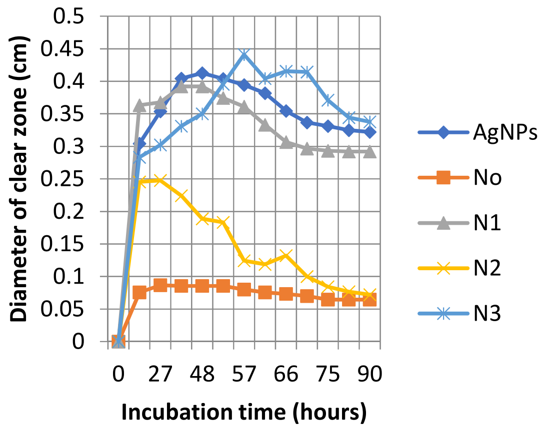

(b)

Figure-4. Antibacterial activity of Nylon cloth against (a) *Staphylococcus aureus* ATCC 25923 and (b) *Escherichia coli* ATCC 35218.

Based on these data indicated that the modifications with the addition of HDTMS compounds shown to increase the antibacterial activity of Nylon fabric deposited silver nanoparticles toward bacteria of *S. aureus* and *E.coli*. The addition of HDTMS compound will not decrease antibacterial activity of the silver nanoparticles coated on the fabrics [22]. ANOVA test based on two factors i.e. the incubation time and the type of sample (Table-4) shows that the significance between diameter of clear zone at the different incubation time against the bacteria *Staphylococcus aureus* ATCC 25923 is 0.000 ($P < 0.05$), meaning there is significant difference between the antibacterial activity at the different incubation time against the bacteria *Staphylococcus aureus* ATCC 25923. In the test between the types of samples used, indicating the significance of 0.000 ($P < 0.05$) which means that there are the differences significantly in antibacterial activity between the types of samples against *Staphylococcus aureus* ATCC 25923. However, for all the difference in the diameter of clear zone at different incubation times and different sample types in inhibiting the growth of *Staphylococcus aureus* ATCC 25923 shows a value of 0.202, and in inhibiting the growth of *Escherichia coli* ATCC 35218 shows a value of 0.423, it means that there are no significant differences in terms of antibacterial activity at different incubation times and different sample types, simultaneously.

Table-4. Two way ANOVA (two factor): type of sample and incubation time against *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 35218.

Source	Df	Mean square	F	Sig.	Conclu-sion
***Escherichia coli* ATCC 35218**					
Time	12	0.057	5.777	0.000	Sig.
Sample	3	0.669	68.291	0.000	Sig.
Time & Sample	36	0.010	1.042	0.423	Not sig.
***Staphylococcus aureus* ATCC 25923**					
Time	12	0.039	5.682	0.000	Sig.
Sample	3	0.914	132.859	0.000	Sig.
Time & Sample	36	0.009	1.238	0.202	Not Sig.

In this study, all samples have antibacterial activity against *S. aureus* and *E. coli*. This can occur because Nylon 6.6 contains amide groups. The amide functional groups can bind or react with silver nanoparticles through covalent coordination. Other possibility is the functional groups of amide presumably can promote the process of silver ion reduction as revealed by [23] that functional groups of aldehyde presumably can promote the process of silver ion reduction (probably analogous to the reaction of "silver mirror").

The Nylon 6.6 coated with silver nanoparticles showed anti-bacterial activity against *Escherichia coli* and *Staphylococcus aureus*. Textiles coated nanoparticle - silver showed antibacterial activity against *Escherichia coli* and *S. aureus* [1, 4, 5-6, 8-9], *Pseudomonas aeruginosa*, *Enterobacteraerogenes*, *Proteus mirabilis*, *K. pneumoniae*, *Candida albicans* yeasts, and micromycetes [23]. The antimicrobial test performed on the treated fabrics against *Staphylococcus aureus* as a gram positive and *Escherichia coli* as a gram negative bacterium showed that a bacterium growth decrease above 96% achieved with 200ppm nano silver with standing up to 20 successive rinses [24].Moreover, silver nanoparticles imparted reasonable antibacterial properties to the cloth against *Staphylococcus aureus* [25].

CONCLUSIONS

Silver nanoparticles can be prepared from a solution of silver nitrate with tri-sodium citrate as a reducing agent and PVA as a stabilizer agent, formed at a wavelength of 429 nm. The adding of HDTMS decreases the absorption intensity of functional groups but increases the contact angle of Nylon 6.6 cloth. Nylon 6.6 cloth - HDTMS has the highest contact angle. There is a significant difference in antibacterial activity among all samples. Nylon 6.6cloth - nanoAg- HDTMShas the highest antibacterial properties. The antibacterial activity of Nylon 6.6 cloth without and deposited nanoparticle-silver against *Staphylococcus aureus* ATCC 25923 and *Escherichiacoli* 32518 are different. All samples have shown a clear zone in inhibiting of growing bacteria *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 35218 at all the incubation time.

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