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Original Article

Antibacterial Activity of Silver Nanoparticles Loaded Fabrics of Nylon and Their Hidrophobicity

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Abstract

The objective of this research is to study antibacterial and hidrophobic properties of nylon 6,6 coated with silver nanoparticlesand hexadecyltrimethoxcysilane (HDTMS). The silver nanoparticle was prepared ~~with~~by chemical reduction method ~~by~~ using tri-sodium citrate as reducing agent and PVA as stabilizer. The silver nanoparticlewas deposited on fabrics ofnylon 6,6 as antibacterial agent and HDTMSwas coated on those as hydrophobic agent. The fabrics of nylon 6,6 were characterized by analyzing the functional groups using ATR-FTIR, hydrophobic properties by measuring contact angle, and antibacterialproperties by measuring clear zone. The addition of HDTMS compound can decreasethe intensity of absorption bands of functional groups but increase hydrophobic property of nylon 6,6. Nylon 6,6 which was coated with silver nanoparticle and HDTMS has the highest antibacterial properties. The antibacterial properties of nylon 6,6before and after modification against *Staphylococcus aureus* ATCC 25923 and *Eschericia 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, and silver nanoparticles.

1. 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 ofmicroorganismsgrowth, 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 beingincreased by 1.16 – 1.32 fold [8]. Silver nanoparticles can be synthesized via chemical reduction method [8] and green synthesis method [9]. $AgNO_3$ solution is used as the base material and trisodium citrate is used as a reducing agent. In addition, the antibacterial propertyies 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 theycan 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]. Superhydrophobicconductive cotton textiles with antibacterial activity were synthesized successfully by in situ coating textiles with AgNPs followed by hydrophobization [12]. This method ~~was~~has beencommonly used to multifunctionalizing conventional textiles with combined properties of superhydrophobicity, antibacteria, andconductivity.

One effort should be made to develop a self-cleaning textile products and antibacteria, by developing a hydrophobic textile material, followed by application of particular nanoparticles effectively and selectively. The nanoparticles can kill many types of microbes in a broad spectrum, but they are not toxic to pathogenic microbes.Textile materials with hydrophobic properties can be obtained by adding silane compound. Addition of silane-based molecules with the surface free energy to be low enough and with the hydrocarbon chain to be sufficient length is one way to make the hydrophobic textile materials [13].In this work, the preparation of silver nanoparticle has been carried out by applying the bottom-up approach using reduction process. Then the textile material was coated with silver nanoparticles. Furthermore, the textile material that has been coated with silver nanoparticles, was then modified by adding HDTMS to produce hydrophobic textile.

2. 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 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, UniversitasGadjahMada.

The tools used included 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 silver nanoparticle(nanoAg)

Preparation of silver nanoparticle 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 under nitrogen atmosphere at~~temperature of~~ 90^0C [14, 15]. Trisodium citrate solution was added dropwise. Heating and flowing of N_2 gas were ~~stopped~~ceasedwhen the solution already transformed intoyellow, but stirring was still done until room temperature reached. Thecolloidal~~of~~silver nanoparticle was characterized using UV-Visspectrophotometer.

Application of silver nanoparticles on nylon 6,6 cloth (Nylon 6,6-nanoAg)

Nylon 6,6 was 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. It was immersed in the colloidal ~~of~~silver nanoparticle then twisted around using a shaker with a speed of 155 rpm for 24 hours. The samples that have been soaked, then dried.

Modification of surface of Nylon 6,6 cloth with HDTMS (Nylon 6,6-HDTMS)

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

The samples which wereprepared in this work werelabellednylon cloth (N0), nylon cloth-nanoAg (N1), nylon cloth-HDTMS (N2), and nylon cloth-nanoAg-HDTMS (N3).

Characterization

The characteristic of silver nanoparticlewas performed using UV-Vis spectrophotometer.The absorbance of silver nitrate solution $1x10^{-3}$ M and silver nanoparticle, were measured using a reference solution of distilled water. The functional

groups of nylon fiber samples before modification, after being coated with silver nanoparticles, with HDTMS, and with silver nanoparticles and HDTMS were analyzed by using Fourier Transform Infrared-Attenuated Total Reflactance spectrophotometer.

The properties ofanti-dirty (hydrophobic) of the samples were determined by measuring the water contact angle (θ) between the fluid and the sample surface [1]. The images were processed using asoftware 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 (NA) and Nutrient Broth (NB) 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 were performed on an agar medium NA and incubated for 24 hours at room temperature. *Staphylococcus aureus* 25923 and *Escherichia coli* ATCC 32518 which have been rejuvenated for 24 hours were then inoculated into a liquid medium NB in the culture bottles and incubated for 24 hours at ~~a temperature of~~ 37^0C. Meanwhile, NA was poured into each petri of approximately 10 mL and allowed to stand for about 24 hours. Each sample was cut with a diameter of 0.5 cm, inserted into the petri dish and allowed in the incubator for 24 hours, then observed the clear zone.

3. Results and Discussion

Figure 1 shows the UV-Vis spectra of the silver nitrate solution and silver nanoparticles.Peak at wavelength of 429 nm indicates the typical absorbtion band of nanoparticles-Ag^0. This is consistent with the works of Barud*et al*. [16], Saputra*et al*.

[14], and Ibrahim [8].The analysis of the functional groups (Table 1) shows that all samples of nylon 6,6 with and without modifications have functional groups of alkyl, C = O, N - H deformation, amides, C - N, C - C stretching, N -H wagging, and C-H rocking. Based on IR-spectra as shown in Figure 2 it can be seen that there ~~was~~is no significant difference among functional groups of Nylon 6,6 (N0), Nylon 6,6-nanosilver (N1), Nylon 6,6-HDTMS (N2), and Nylon 6,6-nanosilver-HDTMS (N3). But the intensity of functional groups of the Nylon 6,6-HDTMS and Nylon 6,6-nanosilver-HDTMSare lower in comparison to Nylon 6,6 (N0) and Nylon 6,6-nanosilver(N1).This is likely because HDTMS compoundwas coatedonto fabric of Nylon 6,6 (N2) as a whole so that the infrared radiation was blocked by the HDTMS compound. Likewisethe intensity of Nylon6,6-nanosilver-HDTMSdecreased. As shown in Fig. 2b, the addition of nanosilvertoward fabrics of Nylon 6,6 ~~did~~does not change the intensity of functional groupsof Nylon 6,6.It seems because the size of the nanoparticles does not significantly affect the structure of the fabric ofNylon 6,6. Nanosilverparticles spread to the fibers and do not cover the surface of the fabrics so that the infrared radiation might notbe transmitted properly.

Table 2 and Figure 3 provide information that the highest contact angle is on a sample of nylon fabric whichwas coated with 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 alkoxide group and a long alkyl chain that has the ability to hold water well [12]. The modification of the fabric with a compound ofHDTMS will produce a rough surface that will reduce the surface free energy and can cause fabric properties to be superhydrophobic[17].Silane compounds

have a characteristic to provide very low surface free energy on the surface of the fabric treated with the compound[18].

The contact angle increasedwith increasing of alkyl chain ofsilane. Alkyl with carbon 16 (C16) can produce the hydrophobic fabric. Hydrophobic properties are due to very rough surface which isformed by a layer of particulate matter [18].The binding between HDTMS compounds with nylon fibers can produce hydrophobic nylon fabrics.The difference ~~between~~of the contact angle ~~of~~on pure nylon cloth ~~with~~o a nylon cloth-HDTMS is so significant. However, the addition of silver nanoparticles decreased the hydrophobic properties of the fabric. It was reported in previous studies that the addition of thesenanosilver particles to hydrophobic materials, such as composite resins, caused an increase in surface free energy and a reduction of the contact angle, which is in accordance with the results of the present study. The addition of 0.5% nanosilver to the composites caused a decrease in the contact angle of water [19].

Antibacterial Activity of Nylon Cloth against *Staphylococcus aureus* ATCC 25923 and *Eschericia coli* ATCC 32518

Antibacterial activity test was performed using *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 35218. The parameters used in this analysis is the diameter of the clear zone that appears at around the sample. The clear zone around the sample was formedby 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 N3 shows the highest antibacterial activity against bacteria *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 35218. Nevertheless, the antibacterial activity of the lowest is on the sample of N0. Overall, the increasing of incubation time can cause increasing of inhibition zone of nylon untill 48 hours incubation, except N0 and N2 showing the decreasing theirsinhibition zone diameter.

The diameter of inhibition zone on all samples tends to decrease after the incubation time of 48 hours except N2 and N3samples. Of the four samples tested, the N3samples (Nylon-AgNPs-HDTMS) showed the highest clear zone. Figure 4 shows the sequence of antibacterial activity of Nylon 6,6 starting lowest to highest is as follow: N0<N2<N1<N3. The lowest antibacterial activity against *Staphylococcus aureus* is sample of N0and the highest antibacterial activity is in the samplesof N3. Samples of N0 showed very low inhibition zone. This is because N0 samples do not have antibacterial properties. Diameter of zone of inhibition of the sample of N2 is not so widegreat because the N2 samples donot haveantibacterial activities. However, the addition of HDTMS in N2 causeshydrophobic properties. The N2 samples show diameter of the inhibition zone to be low. The N1 samples reached the top of the highest inhibition zone diameters at 48th hours and after that the diameter of inhibition zone was unchanged. The highest peak of the diameter of inhibition zone was on samples N3 at the 57th hour.

The silver nanoparticleshave a large surface area so as to facilitate their contact with microorganisms. Silver nanoparticles kill bacteria via the process of diffusion [20]. The study of antibacterial silver nanoparticles was performed by Patel *et al*. [21] which stated that the antibacterial properties of nanoparticles wasassociated with its smallsize,

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 sulfihydryl (-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-Ag is very stable on the cell surface of bacteria because bacteria have sulfihydryl compounds that are not owned by mammals. The silver nanoparticles are not toxic in animals and humans.

These data indicated that the modifications with the addition of HDTMS compound caused to increase the antibacterial activity of Nylon fabric deposited withsilver 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 of *Staphylococcus aureus* ATCC 25923 is 0.000 ($p < 0.05$), meaning there is significant difference ~~between~~of the antibacterial activity at the different incubation time against the bacteria.The test ~~between~~on the types of samples usedindicates the significance of 0.000 ($p < 0.05$) which means that the differences are significant in antibacterial activity ~~between~~on the types of samples against *Staphylococcus aureus* ATCC 25923. However, 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 show~~s~~ a value of 0.202, and in inhibiting the growth of *Escherichia coli*ATCC 35218 it is 0.423, it means that there isno significant differences in terms of antibacterial activity at different incubation times and different sample types.

In this study, all samples showed antibacterial activity against *S. aureus* and *E. coli*. This is because Nylon 6,6 contains amide groups. The amide functional groups can bind or react with silver nanoparticles through covalent coordination. Other possibility is that the functional groups of amide presumably can promote the process of silver ion reduction as revealed by Eremenko et al [23] stating thatfunctional 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 shows antibacterial activity against *Escherichia coli* and *Staphylococcus aureus.* Textiles coated with silver nanoparticleshowed antibacterial activity against *Escherichia coli* and *S. aureus* [1, 4, 5-6, 8-9], *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, *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 decreased above 96% wasachieved with 200ppm nanosilverwithonstanding up to 20 successive rinses [24].Moreover, silver nanoparticles imparted reasonable antibacterial properties to the cloth against *Staphylococcus aureus* [25].

4. Conclusions

Silver nanoparticles can be prepared from a solution of silver nitrate with tri-sodium citrate as a reductant agent and PVA as a stabilizer agent. The addition 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 6,6 cloth-HDTMS-nanosilver has the highest antibacterial properties. The antibacterial activity of nylon cloth without and loadedsilver nanoparticle against *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* 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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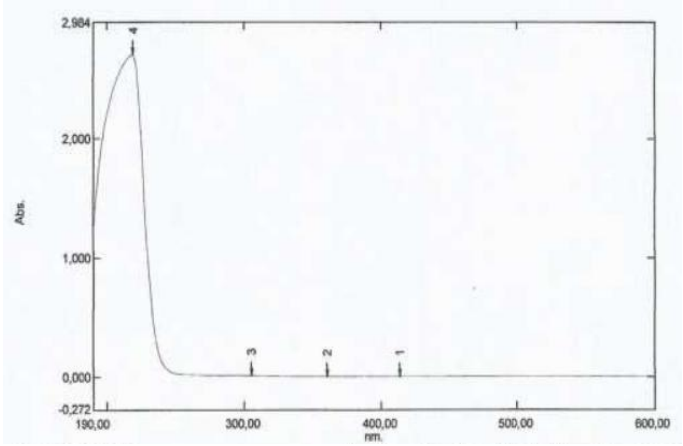
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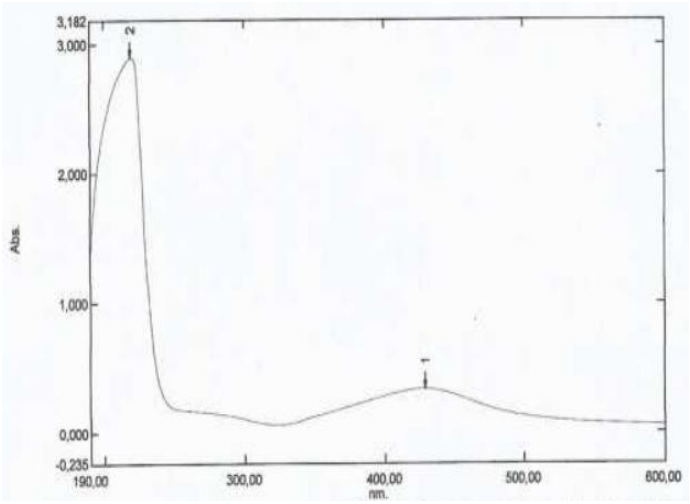
2,000
1,000
0,000
-0,272
Abs.
190,00
300,00
400,00
500,00
600,00
nm.
4
3
2
1

(a)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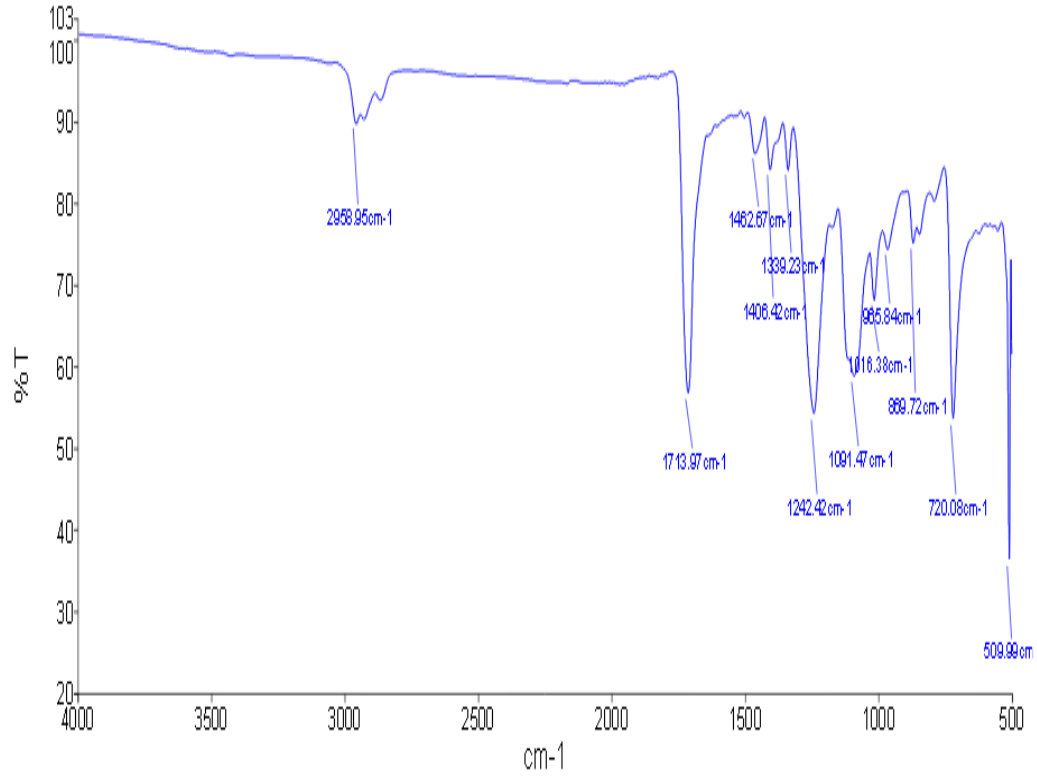
3,000
2,000
1,000
0,000
-0,235
Abs.
190,00
300,00
400,00
500,00
600,00
nm.
2
1

(b)

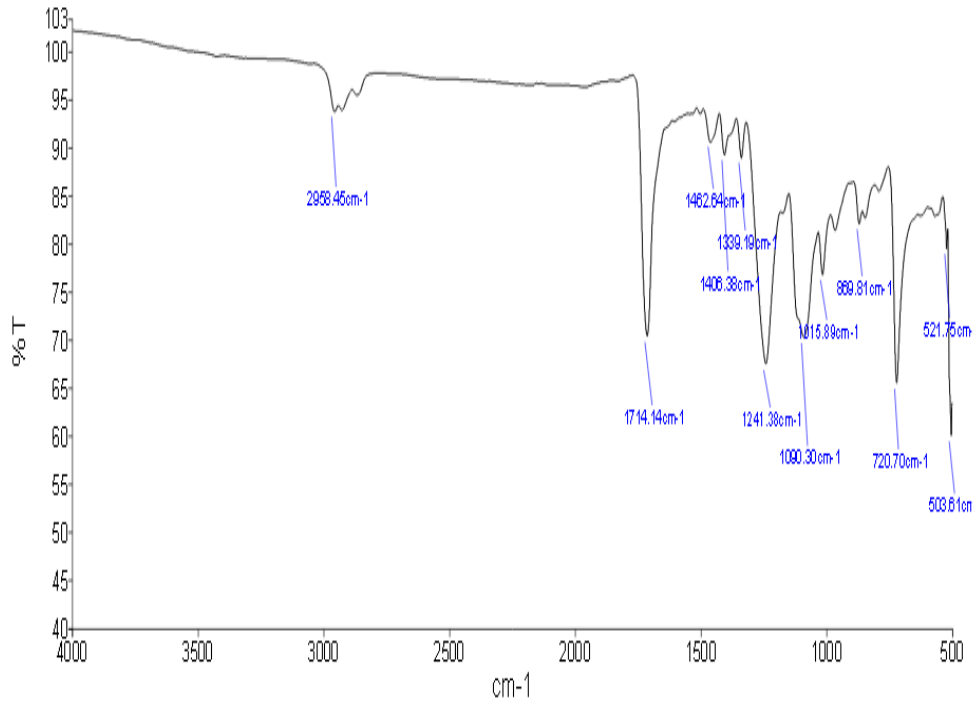

500 ml
IWAKI
PYREX
500
400
300
200
100

(c)

Figure 1 The spectra Uv-Vis ofthe silver nitrate solution(a), silvernanoparticle(b), and AgNPs colloidal(c).



(a)


%T
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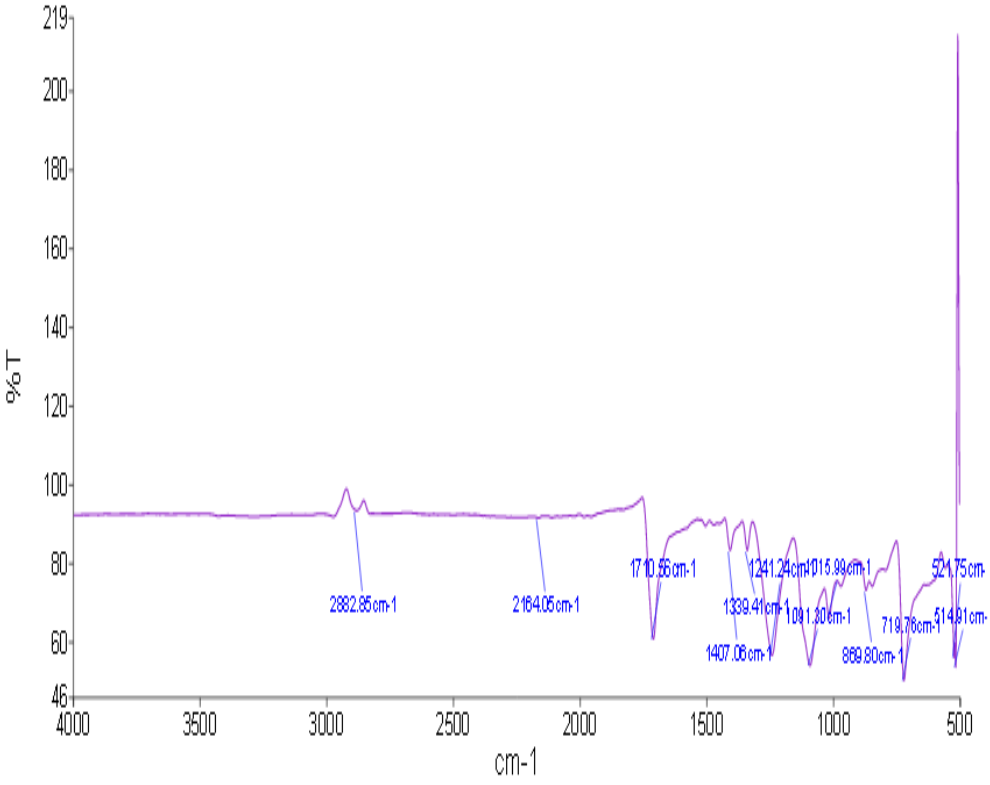
2958.45cm-1
1714.14cm-1
1462.64cm-1
1406.38cm-1
1339.19cm-1
1241.38cm-1
1090.30cm-1
869.81cm-1
720.70cm-1
503.61cm

(b)


%T
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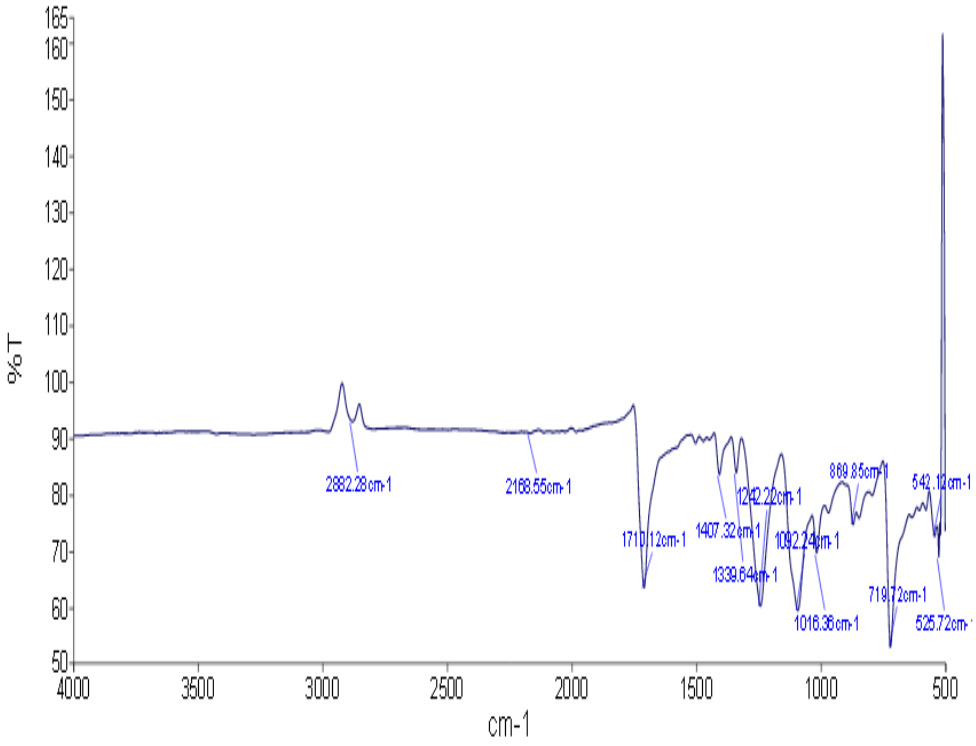
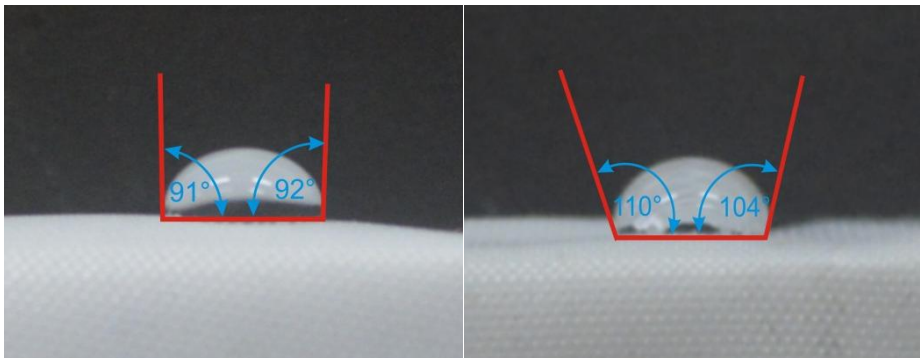
2164.05cm-1
1710.56cm-1
1407.06cm-1
1339.41cm-1
1241.24cm-1
1091.30cm-1
1015.99cm-1
869.80cm-1
719.76cm-1
521.75cm
514.81cm

(c)

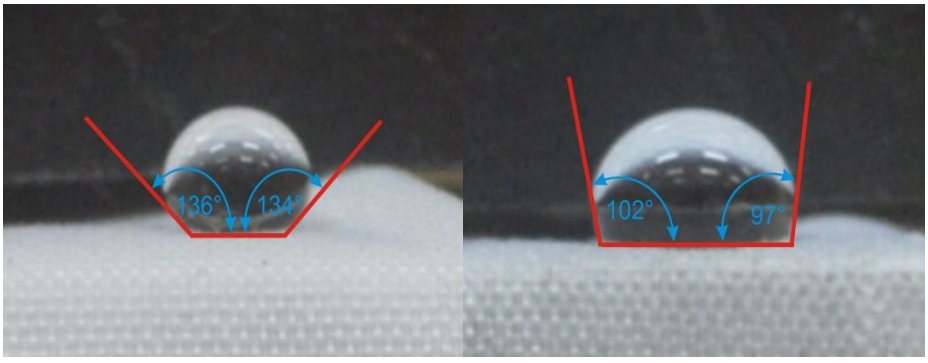


(d)

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

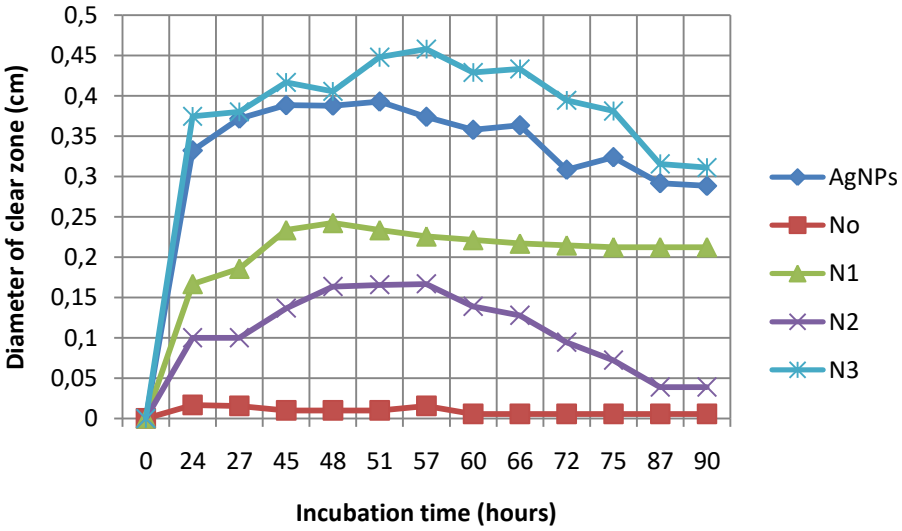


(a) (b)

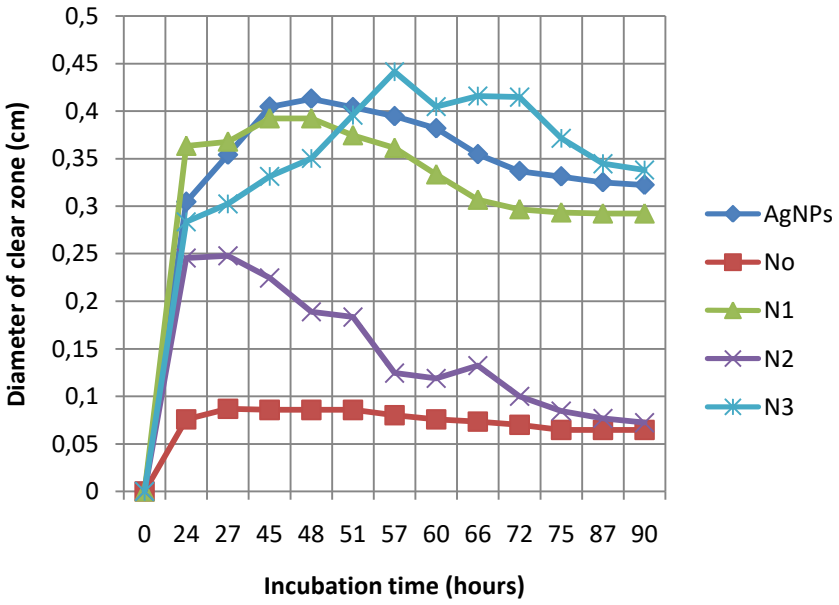


(c) (d)

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



(a)



(b)

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

No	Interpretation of Functional Groups	Wave number (cm^{-1})			
		N0	N1	N2	N3
1	C=O stretching	1713.97	1714.14	1710.56	1710.12
2	Alkyl	2958.95	2958.45	2882.85	2882.28
3	C-N	1242.42	1241.38	1241.24	1242.22
4	N-H deformation	1462.67	1462.64	1407.06	1407.32
5	Amide	1339.23	1339.19	1339.41	1339.64
6	C-C stretching	1016.38	1015.89	1015.99	1016.36
7	N-H wagging	869.72	869.81	869.8	869.85
8	CH_2 rocking	720.08	720.7	719.76	719.72

Table 1 Functional groups of Nylon6,6 before and after modification

Sample Contact Angle	Nylon	Nylon-Ag	Nylon-HDTMS	Nylon - Ag-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

Table 2 Contact angle of nylon before and after modification.

Incubation Time (Hours)	Average diameter of the clear zone (mm)							
	Staphylococcus aureus				*Escherichia coli*			
	N_0	N_1	N_2	N_3	N_0	N_1	N_2	N_3
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

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

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.	Conclusion
Escherichia coli ATCC 35218						
Time	0.679	12	0.057	5.777	0.000	Significant
Sample	2.008	3	0.669	68.291	0.000	Significant
Time & Sample	0.368	36	0.010	1.042	0.423	Not significant
Staphylococcus aureus ATCC 25923						
Time	0.469	12	0.039	5.682	0.000	Significant
Sample	2.741	3	0.914	132.859	0.000	Significant
Time & Sample	0.307	36	0.009	1.238	0.202	Not Significant

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