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Silver Nanoparticle-Graphene Oxide Mixture as Anti-Bacterial Against *Staphylococcus aureus*

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Abstract. This study aims to i) synthesize silver nanoparticle-graphene oxide (Nano-Graver) mixture via liquid sonication exfoliation method using graphite from 2B pencil, ii) characterize the Nano-Graver solution based on UV-Vis, FTIR, XRD, and SEM, and iii) determine the Nano-Graver solution inhibition zone diameter against *Staphylococcus aureus* (*S. aureus*) bacteria. This is an experimental research with steps given as follows: i) synthesizing graphene oxide (GO) via the liquid sonication exfoliation (LSE) method, ii) synthesizing silver nanoparticles (AgNPs) chemically from $AgNO_3$, iii) mixing the GO and AgNP solutions to become Nano-Graver, iv) characterizing the Nano-Graver solutions, and v) determining the Nano-Graver solution inhibition zone diameter against *S. aureus* bacteria via nutrient agar (NA) and blood agar (BA) media. The Nano-Graver solution is successfully synthesized having a dark-yellowish color, compared to a yellowish color solution of the AgNP and a clear solution of GO. The UV-Vis test of the Nano-Graver solution shows two absorbance peaks at wavelengths of 230 nm and 435 nm indicating the existence of GO and AgNP materials, respectively. The FTIR result shows OH and C = C groups, which means GO material occurs in the Nano-Graver solution. The XRD test shows that the Nano-Graver material has a semi-crystalline or amorphous structure. The SEM test for the Nano-Graver solution shows GO in coral-like material decorated by small spheres of AgNPs. The inhibition zone test of *S. aureus* bacteria produces the largest inhibition zone diameters in NA and BA media of 1.731 cm and 1.043 cm, respectively, for Nano-Graver concentration of 5 mM.

INTRODUCTION

Staphylococcus aureus (*S. aureus*) is pathogenic bacteria that may cause infections during treatment or post-surgery in a hospital due to less or poor sterile conditions. These infections call nosocomial diseases and can cause mortality [1]. World Health Organization (WHO) study shows that this disease has affected around 8.7% of patients treated in hospitals in 14 countries in the world. Moreover, this infection can cause complications, such as sepsis, pneumonia, endocarditis, osteomyelitis, meningitis, and toxic syndrome [2]. Therefore, a solution is needed to inhibit the growth of *S. aureus* bacteria so that nosocomial disease can be prevented.

Nanotechnology is an interdisciplinary field of study, which is related to the synthesis, design, and engineering of materials in the scales between 1 nm to 100 nm [3]. Nanomaterials have chemical and physical properties that are far superior to the bulk materials. This is because nanomaterials have far more surface contact areas that can increase their chemical reactivity. One of the nanomaterials that are currently being studied is a combination of silver nanoparticles (AgNP) and graphene oxide (GO). AgNP-GO nanomaterials, or in this case we call it Nano-Graver, can be used for various applications, such as for biosensor and intracellular drug delivery [4], carbon dioxide reduction [5], ambipolar field-effect transistor (FET) [6], as a catalyst [7], and as antibacterial [8,9]. The antibacterial activities of AgNPs are inactivating the phosphomannoseisomerase enzyme and cause bacterial lysis

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[10]. However, one weakness of AgNPs is aggregation. This aggregation decreases the effectiveness of the anti-bacterial. Hence, it is necessary to add GO as a stabilizer in the AgNP solution.

GO is commonly synthesized via the (modified) Hummer's method [11]. However, another technique for synthesizing GO is liquid exfoliation (LE) using a blender [12] or ultra-sonication [13]. The advantages of GO synthesized using the latter or liquid sonication exfoliation (LSE) are affordable, efficient, and easy to operate on a large scale [14]. Therefore, in this study, the GO is synthesized using the LSE method by utilizing a primary material of graphite obtained from 2B pencils. Moreover, the synthesis of AgNP is carried out chemically from $AgNO_3$ powder. Furthermore, the Nano-Graver solution that has been synthesized is expected to inhibit bacteria *S. aureus*.

EXPERIMENTAL

This is experimental research. The subject of this study is the Nano-Graver solution and the object is the *S. aureus* bacteria. Experiments have been carried out through the synthesis of AgNP, GO, and Nano-Graver solutions. Furthermore, the inhibition zone of the Nano-Graver solution against *S. aureus* bacteria is determined to test the anti-bacterial property of the Nano-Graver.

The tools that are used in this study are (a) a piezoelectric sonicator, (b) UV-Vis spectrophotometer (Shimadzu UV-2550), (c) FTIR spectrometer (Nicolet Avatar), (d) XRD (Rigaku Miniflex 600), (e) SEM (CoXem), (e) an ultrasonic homogenizer, (f) a laminar automatic flow (LAF), (g) an autoclave, (h) a digital scale, (i) a magnetic stirrer, (j) a paper disc, (k) a caliper, and (l) a blender. The Materials used in this study are (a) graphite powder from 2B pencils, (b) $AgNO_3$ powder, (c) sodium citrate, (d) nutrient booth (NB), (e) nutrient agar (NA), (f) blood agar (BA), (g) chloramphenicol solution, (h) distilled water, (i) alcohol 70%, and (j) cotton buds.

(a) (b) (c)

FIGURE 1. GO synthesis process. Graphite powder solution after blending (a), the piezoelectric sonicator (b), and the sonication process (c).

The work steps in this study are explained as follows:

- **GO synthesis**. i) Weighing 1 gr of graphite powder from 2B pencils, ii) dissolving the graphite powder in 300 ml distilled water, iii) stirring the solution using a blender for 3 minutes [Figure 1(a)], iv) performing ultra-sonication towards the solution [Figure 1(c)] with ultrasonic frequency [Figure 1(b)] of 32 kHz for 5 hours, and finally v) leaving the solution to equilibrate for one night.
- **AgNP synthesis**. AgNP is synthesized by reducing $AgNO_3$ using sodium citrate. i) Weighing $AgNO_3$ powder as much as 0.425 gr, ii) dissolving the $AgNO_3$ powder into 500 ml of distilled water, iii) taking and steering 10 ml $AgNO_3$ solution and heating it for 10 minutes [Figure 2(a)], iv) removing the $AgNO_3$ solution from the heating source and adding 3 drops of sodium citrate with a concentration of 1% into the solution, and finally v) reheating the solution until the sample becomes yellowish color [Figure 2(b)]. The yellowish color is an indication that $AgNO_3$ has been reduced to AgNP.
- **Nano-Graver synthesis**. Nano-Graver is synthesized by mixing 10 ml of 5 mM AgNP solution with 3 ml GO solution in a beaker glass. Then the mixture is homogenized and heated with a magnetic stirrer for 30 minutes.

- **Characterizations of Nano-Graver**. After obtaining the Nano-Graver solution, characterizations are carried out using four instruments, i.e.: UV-Vis, FTIR, XRD, and SEM. The UV-Vis and FTIR tests are carried out in liquid phase, while XRD and SEM tests are in the solid phase.
- **Anti-bacterial test (inhibition zone)**. Anti-bacterial testing of the Nano-Graver solution begins with the preparation of *S. aureus* bacteria in NA media. Then the bacteria are grown in NB liquid media for 24 hours and then planted on 1 ml NA and BA solid media with a micropipette. Paper disks are given in a bag that has been soaked in 5 mM of AgNp and Nano-Graver solutions. The control and positive test paper disks are given by soaking them in distilled water and chloramphenicol, respectively. The anti-bacterial test data is analyzed using statistical analysis in the form of Annova and Duncan tests [15].

(a) (b)

FIGURE 2. Heating the $AgNO_3$ solution (a) and the AgNP solution formed (b).

RESULTS AND DISCUSSION

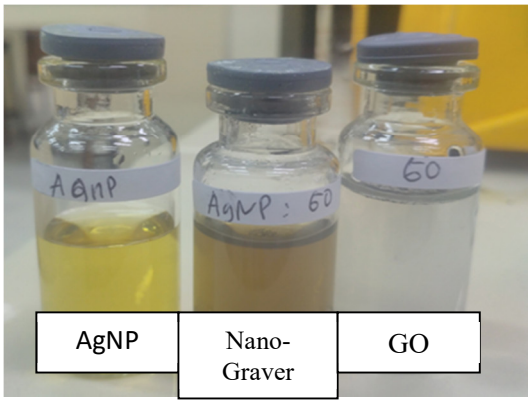

FIGURE 3. The solutions obtained in this study, i.e.: AgNp, Nano-Graver, and GO.

Various solution samples are produced from this study, namely AgNP, Nano-Graver, and GO (Figure 3). It can be physically observed from Fig. 3 that the AgNp solution is yellowish color, while GO solution looks clear. However, a combination of AgNP and GO becomes Nano-Graver with a dark yellow colored solution. The various tests have been conducted using UV-Vis, FTIR, XRD, and SEM to determine the characteristics of the Nano-Graver solution.

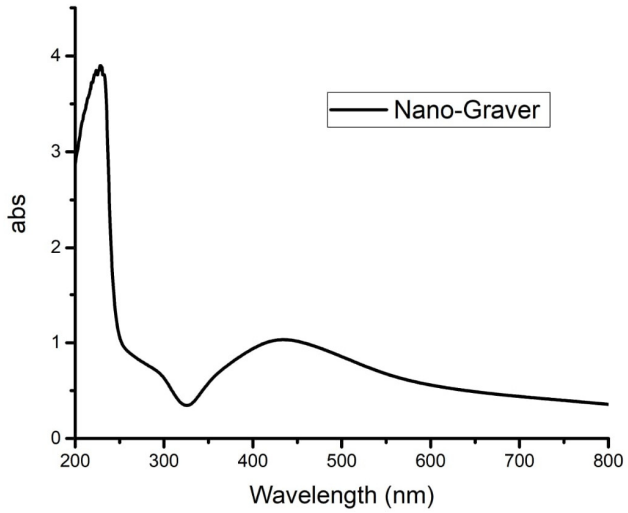

FIGURE 4. UV-Vis characterization result of Nano-Graver solution with a concentration of 5 mM.

UV-Vis test is used to determine the absorbance peaks of the Nano-Graver solution. The UV-Vis test result for the Nano-Graver solution can be observed in Fig. 4. Fig. 4 shows two absorbance peaks and one shouldering peak. The first and shouldering peaks are at wavelengths of 230 nm and 300 nm, which show the existence of GO [16]. The first peak at 230 nm represents π - π* transitions and indicates the presence of C = C bonds in the solution, whereas the shouldering peak at 300 nm represents n - π* transitions and indicates the existence of oxygen bonds in the solution. The second peak is located at a wavelength of 435 nm, which indicates the presence of AgNp [17]. These two absorbance peaks show that the synthesis of Nano-Graver material has been successfully carried out.

The FTIR characterization is used to determine the functional groups existing in the Nano-Graver solution. The FTIR result can be observed in Fig. 5. FTIR test result for the Nano-Graver solution shows that there is a transmittance band (peak) at a wavenumber 3440 cm^{-1}, which indicates the presence of OH (hydroxyl) group. This hydroxyl group indicates the presence of oxides in GO [16], which is in accordance with the n - π* electronic transitions in the UV-Vis result. A transmittance peak at a wavenumber of 1633.48 cm^{-1} shows the existence of C = C functional groups, which is consistent with the existence of the first peak at 230 nm in the UV-Vis result. The C = C functional groups are the basic structure of GO and Nano-Graver materials.

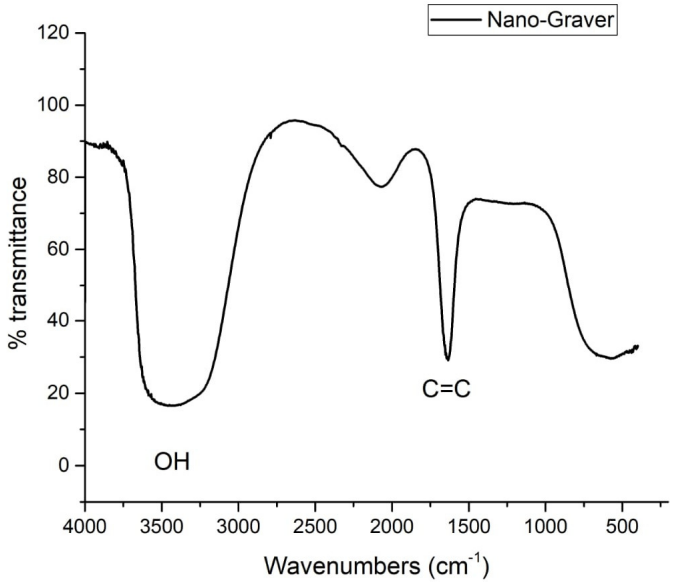

FIGURE 5. FTIR characterization result for the Nano-Graver solution with a concentration of 5 mM.

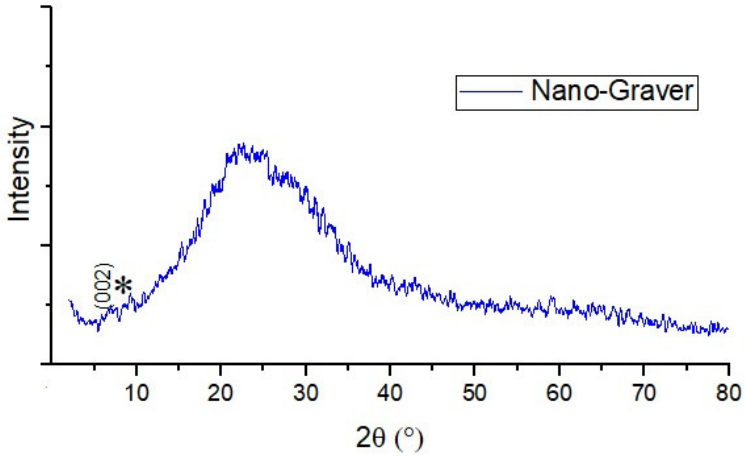

FIGURE 6. XRD characterization result of Nano-Graver material.

XRD characterization is performed to determine the crystal structure of the Nano-Graver material in the solid phase. The XRD result is shown in Fig. 6. An indication of AgNp material is represented by a peak at 2θ of 31.05° with hkl plane of (113) in accordance with a peak in the XRD result of AgNp material obtained in [18]. Furthermore, the GO material is indicated by peaks of 2θ with values of 9.07° and 28.13°, which is in accordance with a study in [19]. The XRD test result for the Nano-Graver solution did not show typical sharp and high peak intensities. This indicates that the Nano-Graver solution has a semi-crystalline or amorphous structure.

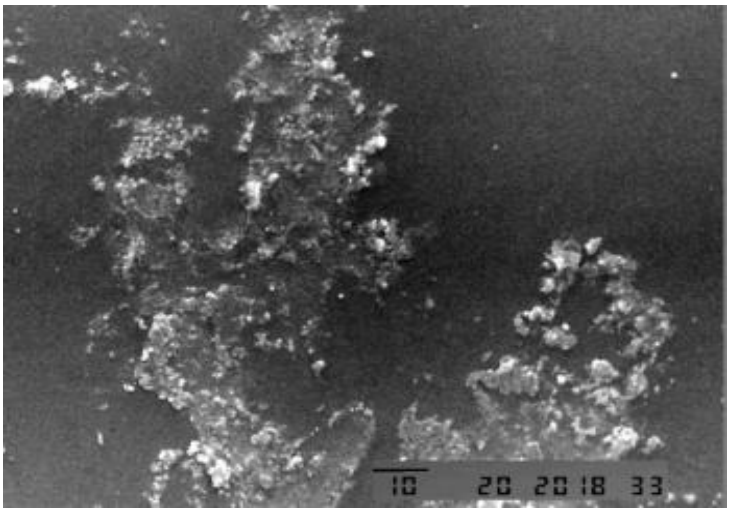

FIGURE 7. The SEM result of the Nano-Graver with 1000X magnification

Finally, Figure 7 shows the result of the SEM test for Nano-Graver solution. The SEM result shows that the Nano-Graver solution has a mean particle size of 44.72 nm. It can be observed from Fig. 7 that the Nano-Graver material has a surface morphology in the form of coral-like materials. These coral-like materials represent GO and decorated by small-spheres indicating the AgNp particles.

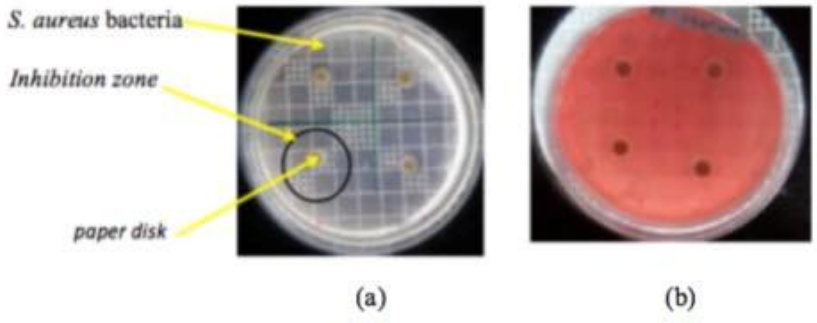

FIGURE 8. The test results of *S. aureus* bacteria inhibition zone on (a) NA and (b) BA media.

The AgNp and Nano-Graver solutions as anti-bacterial are tested in two media, namely on NA [Figure 8(a)] and BA [Figure 8(b)] media. Figure 8 shows the inhibition zones of *S. aureus* bacteria, which is characterized by clear areas on NA and BA media. Measurements of the inhibition zone diameter uses a digital caliper, and the results can be observed in Table 1.

TABLE 1. Results of the inhibition zone diameters

Solution	Distilled Water	Chloramphenicol	AgNp			Nano-Graver		
			1 mM	3 mM	5 mM	1 mM	3 mM	5 mM
Average inhibition zone diameter ± 0.001 (cm)	0.000	1.553	0.946	0.925	1.256	0.927	1.002	1.731

The statistical analysis [15] indicates that there is a significant effect of the treatment of Nano-Graver solution on *S. aureus* growth inhibition. The Duncan analysis with a confidence level of 95% proves that there are differences in the treatment effect of varying concentrations of the growth inhibition zone diameter of *S. aureus* bacteria. The inhibition zone diameter of Nano-Graver solution with a concentration of 5 mM shows no significantly different from the positive control of chloramphenicol. For distilled water, the diameter of the inhibition zone is 0.00 cm, which means that there is no inhibition of the growth of *S. aureus* bacteria. The use of NA medium proves that the *S. aureus* bacteria can be inhibited by the Nano-Graver solution with the largest inhibition zone diameter of 1.731 ± 0.001 cm.

The Nano-Graver solution is also tested on BA medium. The use of the BA medium also shows that the *S. aureus* bacteria is inhibited using the Nano-Graver solution with the best inhibition zone diameter of 1.043 ± 0.001 cm. Nano-Graver testing on BA medium does not damage the BA structure, and Nano-Graver solution can inhibit bacteria *S. aureus*. Thus, the Nano-Graver solution can be used as an anti-bacterial on the hands and cleaning of medical devices in hospitals that are effective for inhibiting *S. aureus* bacteria.

CONCLUSIONS

The Nano-Graver solution has been successfully synthesized resulting in a dark yellowish colour solution compared to the yellowish solution of the AgNP and clear solution of the GO. The SEM image of the Nano-Graver material shows coral-like materials of GO decorated with small-spheres of AgNP and average particle size of 44.72 nm. The XRD result shows that the Nano-Graver has a semi-crystalline or amorphous structure. The FTIR result shows that the Nano-Graver solution contains C = C and OH functional groups. Finally, the *S. aureus* bacterial inhibition zone diameter of the Nano-Graver solution in NA and BA media are 1.731 ± 0.001 cm and 1.043 ± 0.001 cm, respectively.

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