Distribution of Silver (Ag) Nanoparticle in PVA/Ag Nanofiber Fabricated by Electrospinning Method

Sunaryono¹,²,*, Aulia Rachmawati¹, Elya Khunazatus Shima³, Ahmad Taufiq¹,², Hendra Susanto³, Nandang Mufti¹,², Sujito⁴, Sugiharto⁴, Munasir⁵,⁶

¹Department of Physics, Faculty of Mathematics and Natural Sciences, Universitas Negeri Malang, Jalan Semarang 5 Malang 65145, Indonesia
²Research Center of Minerals and Advanced Materials, Faculty of Mathematics and Natural Sciences, Universitas Negeri Malang, Jl. Semarang 5 Malang 65145
³Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Malang, Jl. Semarang 5 Malang 65451, Indonesia
⁴Department of Sports Science, Faculty of Sports Science, Universitas Negeri Malang, Jl. Semarang 5 Malang 65145, Indonesia.
⁵Department of Physics, Faculty of Mathematics and Natural Science, Universitas Negeri Surabaya, Kampus Ketintang, Jl. Ketintang, Surabaya, 60231.
⁶Research Center of Advanced Materials, Universitas Negeri Surabaya, Kampus Ketintang, Jl. Ketintang, Surabaya 60231.

*Corresponding author’s email: sunaryono.fmipa@um.ac.id

Abstract. This research has successfully fabricated Ag nanoparticles and PVA/Ag nanofiber as antibacterial agents. The silver nanoparticles were synthesized using a chemical reduction method, while PVA/Ag nanofiber was successfully fabricated with silver nanoparticles and PVA polymer materials using electrospinning method. The chemical reduction method is an effective method for obtaining nanometer-sized silver particles, while electrospinning is one of the most efficient and inexpensive methods to produce nanofiber products. The silver nanoparticles and PVA/Ag nanofiber were characterized using instruments of XRD, SEM, EDX, and antimicrobial test for PVA/Ag nanofiber. In addition, through an X-ray diffraction characterization, the average size of silver nanoparticles was approximately 19.12 nm. Meanwhile, the SEM morphological analysis of PVA/Ag nanofiber has an average fiber diameter of 449.11 nm. The result of EDX analysis of PVA/Ag nanofiber showed the aggregation of silver nanoparticles in the range of polymer fibers shown by white clumps. In addition, the antibacterial test results on PVA/Ag nanofiber within 24 hours of incubation showed that the largest nanofiber zone of inhibition was in the diameter range of 0.9 cm. This case shows that PVA/Ag nanofiber has a relatively low antimicrobial power. This condition can be increased by reducing the possible impact of contamination from PVA/Ag nanofiber by the surrounding environment. Thus, the PVA/Ag nanofiber resulted in this study has a potential to be used as a wound dressing under optimal conditions.

Keywords: Silver, PVA, electrospinning, nanofiber.
1. Introduction
Nanoscience is a science that studies the nature of matters with the size range of 1-100 nm [1]. Meanwhile, nanotechnology is a technique of manipulating atoms and molecules to create new materials and structures at the nanoscale (1-100 nm) with new properties [2]. Nanotechnology involves the process, separation, deformation of the material by one atom or one molecule [3]. The material in the form of nanoparticles has unique properties, which can be controlled and modified in size, shape, chemical properties, and surface functionalization [4]. Nanomaterials have diverse applications in areas such as electronics, medicine, health, energy, biotechnology, information technology, agriculture, environmental food, and others [2]. Metal nanoparticles, such as gold, silver, iron, zinc, and metal oxides as well as multi-walled carbon nanotube (MWCNT)-magnetite nanocomposites have great opportunities in biomedical applications due to their large surface area to volume ratio [5,6].

The studies on one-dimensional nanostructured materials such as nanofiber, nanorod, nanobelt, and nanotubes have been reported by Xinxing et al. [7]. Nanofiber is identified as a very fine fiber with a very small diameter (below 100 nm) [8]. Producing nanofiber can be done with several techniques or methods, one of them is electrospinning method which is a relatively easy, simple, and effective method to produce nanofiber [9] starting from the submicron diameter to nanometer diameter by using a high electric field potential [9], [10]. Each type of nanofiber can be produced from various types of polymers including natural and synthetic polymers. The polymers used to produce the nanofiber generally have good characteristics thus have a potential as filtration, optical fiber, pharmaceutical delivery systems, medical scaffolding network, and protective clothing [11].

Silver nanoparticles have been extensively studied for their unique physical, chemical, and microbial properties, especially in the fields of optics, catalysts, and biomedicine [12]. The silver nanoparticles synthesized by using the Tollens method and dextrin as the stabilizers have a uniform morphology and particle size distribution as well as having the function as antimicrobials [13]. The Ag/PVA nanofiber composites produced by electrospinning technique have an average diameter of 13.8 nm as an antimicrobial agent [14]. The PVA/AgNPs nanofibers produced by electrospinning technique have a nanofiber range size of 135-160 nm, while the size of silver nanoparticles (AgNPs) are ranged between 15 to 27 nm, in which the results can be used for wound dressing components, protective coatings, biomedical devices, and water purification [15]. However, research on the distribution of silver nanostructures in PVA nanofiber is rarely conducted, this study focused on the characterization of silver nanoparticles in PVA nanofiber and antibacterial characterization that have potential applications as wound dressings.

2. Materials and Methods
The materials used for this research were silver nitrate (AgNO₃), sodium borohydride (NaBH₄), polyvinyl alcohol (PVA), polyvinyl pyroledone (PVP), 96% alcohol, and aquades. In the initial stage, the oxygen PVP (first solution) was reacted with 475 g of PVP and then put into 475 ml of aquades and NaBH₄ solution (second solution) by adding 0.433 g NaBH₄ and incorporated into the 25 mL of PVP solution. And then, 0.196 gram of AgNO₃ was incorporated into the first solution, stirred with a magnetic stirrer until became a homogeneous solution. The temperature was kept between 5 to 10° C. Then, the deposition of the solution was put in the oven at a temperature of 100 ° C. After that, the characterization was done by using XRD.

After the Ag nanoparticles were formed, the next stage to be conducted was to produce a PVA/Ag nanofiber with an electrospinning technique. 0.125 gr of Ag nano was incorporated into 1 ml of aquades stirred with a magnetic stirrer until homogeneous. Next, the 0.5 gr of PVA was incorporated into 3.375 ml of aquades stirred with a magnetic stirrer at a speed of 700 rpm and a temperature of 120° C. Subsequently, the Ag nano solution, and PVA solution were then mixed, stirred with a magnetic stirrer. Furthermore, the formed solution was inserted into the ultrasonic cleaner for 2-3 hours. The solution
was put into the syringe and then the process of electrospinning was conducted. After the nanofiber was formed, it was followed by morphological characterization using SEM and antibacterial activity test.

3. Results and Discussion

3.1. Silver (Ag) Nanoparticles

X-ray diffraction results of Ag nanoparticles are shown in Figure 1.

![Figure 1. The Peak of X-Ray Diffraction of Ag Nanoparticles](image)

Based on the diffraction pattern of Figure 1, the peaks that appear are located in the planes 111, 200, 220, and 311. The highest peak is in the (111) plane with the diffraction angle (2θ) between 36.76 °- 39.02 °. This diffraction pattern is in accordance with previous research which has been done by Zhang et al. [14] which states that the highest peak lies at an angle of 38.2°. In order to find out the particle size of the silver nanoparticle sample, the fittings were conducted on the highest peaks of Figure 1. The parameters obtained from the fitting results are calculated into the Scherrer equation to obtain the value of the crystal size. The following Scherrer equations are used.

$$D = \frac{k \lambda}{\beta \cos \theta}$$  

Where:
- $k$ = constant of Scherrer (0.9)
- $\lambda$ = length of radiation wave of Cu Kα (1.5406 Å)
- $\beta$ = FWHM (radian)
- $\theta$ = Bragg Angle (radian)

From the highest peak analysis results from X-ray diffraction data of silver nanoparticles using equation 1, a particle size of 19.12 nm was obtained. The results of different particle sizes shown in other studies were the obtained size of nanosilver particles which were 13.8 nm [14] and 15-27 nm [15].
3.2. Results of SEM-EDX characterization on PVA/Ag Nanofiber

The morphology of the PVA/Ag nanofiber characterized by SEM instrument is shown in Figure 2.

![SEM Morphology of PVA/Ag Nanofiber](image1)

As seen from the result of SEM characterization with a 20,000x magnification, the morphology of the PVA/Ag nanofiber produced has an average diameter size of 449.11 nm. The results obtained in this study were quite large compared to the previous research that has been done by Tan et al. [15] which obtained a nanofiber diameter of 135-160 nm. The difference of the diameter resulted depends on several parameters during the electrospinning process, among which were the properties of polymer solutions such as a solution concentration, molecular weight, viscosity, surface tension, dielectric constant, and conductivity [14,15].

The EDX characterization of the PVA/Ag nanofiber sample was performed to determine the percentage of silver nanoparticles contained. The result of EDX PVA/Ag nanofiber characterization is shown in Figure 3.

![The Result of SEM-EDAX PVA/Ag Nanofiber](image2)

Figure 3. The Result of SEM-EDAX PVA/Ag Nanofiber
Figure 3 shows an EDX characterization of a PVA/Ag nanofiber sample at a particular point marked with a plus sign (+) of red. The percentage of silver nanoparticle content obtained in Figure 3a is 5.68 wt% while in Figure 3b the percentage is 12.24 wt%. The result in Figure 3b was larger because at that point the silver nanoparticles underwent agglomeration indicated by white clumps. The similar study conducted by Lin et al. [16] showed an EDX analysis of several samples of nanofiber PVA/Ag with a nanoparticle content about 2.6 wt% and 12.4 wt%. The higher particle content of Ag in the PVA/Ag nanofiber causes the resulted diameter to be greater [17,18].

3.3. The Result of the Antibacterial Test of PVA/Ag Nanofiber
The results of the antibacterial test performed on the PVA/Ag nanofiber shown in Table 1 with the bacteria used for testing were *Escherichia coli* and *Staphylococcus aureus*.

| Bacteria                     | Repetition | Diameter of Resistor Zone (cm) |
|------------------------------|------------|-------------------------------|
| *Escherichia coli*           | 1          | 0.80                          |
|                              | 2          | 0.90                          |
|                              | 3          | 0.70                          |
| *Staphylococcus aureus*      | 1          | 0.70                          |
|                              | 2          | 0.70                          |
|                              | 3          | 0.70                          |

The antibacterial test was conducted in 24 hours of incubation time. The antibacterial activity test on PVA/Ag nanofiber was performed on two types of bacteria, *Escherichia coli* (gram-negative) and *Staphylococcus aureus* (gram-positive). Based on the results obtained, PVA/Ag nanofiber could cause the zone of inhibition against the growth of colonies of *E. coli* and *S. aureus* bacteria. The zone of inhibition diameter resulted varied and was based on the measurement results, the largest zone of inhibition was present in the test of *Escherichia coli* (see Table 1).

Table 1 shows that the PVA/Ag nanofiber produces larger inhibitory diameters on *Escherichia coli* bacteria compared to the inhibitory diameters of *Staphylococcus aureus* bacteria. In the 24-hour incubation time, the largest zone of inhibition was only 0.9 cm in diameter, it can be said that the PVA/Ag nanofiber has low rates of antimicrobial resistance.

Different results of study are presented by Kong and Jang [19] in their research on the bioactivity of nanocomposite antibacterial performance against E. Coli bacteria showed that the presence of silver nanoparticles in the composite was effective in killing and inhibiting the growth of *E. coli* bacteria, besides the composites studied using methyl acrylate and Ag nanoparticles have been proven to have antibacterial power. The antibacterial characteristics of the silver nanoparticle composites are closely related to the properties of silver nanoparticles that have antibacterial properties [20,21]. The differences in the results of this study with previous studies are due to the time interval of PVA/Ag nanofiber synthesis with antimicrobial tests was too long, allowing the PVA/Ag nanofiber to be contaminated and reduce its antibacterial power. Thus, it needs to be re-tested to ensure the fact about the antibacterial power of PVA/Ag nanofiber.

4. Conclusion
This research concluded that the result of crystal size analysis from the Ag nanoparticles obtained was 19.12 nm. The morphological analysis of PVA/Ag nanofiber using SEM has an average diameter of 449.11 nm. Meanwhile, EDAX analysis of PVA/Ag nanofiber indicated that the aggregation of silver nanoparticles in the range of polymer fibers was characterized by white clumps. The results of the antimicrobial testing on the PVA/Ag nanofiber in the 24-hours of incubation period resulted in a 0.9 cm diameter zone of inhibition, it can be said that the PVA/Ag nanofiber has low rates of antimicrobial power. This is due to the time interval between producing the PVA/Ag nanofiber with the antimicrobial test was too long, thus allowing PVA/Ag nanofiber to be contaminated and reduce its antibacterial power.
power. In sum, the potential of the PVA/Ag nanofiber produced is as an antibacterial agent which can be applied as a wound dressing.

Acknowledgments
The authors would like to express our deep gratitude for the financial support given by PSNI of KEMENRISTEKDIKTI for SN.

References
[1] de Mello Donegá C 2014 Nanoparticles: Workhorses of Nanoscience (Springer)
[2] Prasad T N V K., Subba Rao Kambala V and Naidu R 2011 A critical review on biogenic silver nanoparticles and their antimicrobial activity Curr. Nanosci. 7 531–544
[3] Elumalai E K, TNVKV P, Nagajothy P C and David E 2011 A bird’s eye view on biogenic silver nanoparticles and their applications Chem Sin 2 88–97
[4] Nagarajan R and Hatton T A 2008 Nanoparticles: synthesis, stabilization, passivation, and functionalization (ACS Publications)
[5] Prasad S B, Aeri V and others 2013 Current Understanding of Synthesis and Pharmacological Aspects of Silver Nanoparticles Am. J. Phytomedicine Clin. Ther. 1 536–547
[6] Rahmawati R, Melati A, Taufiq A, Sunaryono, Diantoro M, Yuliarto B, Suyatman S, Nugraha N and Kurniadi D 2017 Preparation of MWCNT-Fe3O4 Nanocomposites from Iron Sand Using Sonochemical Route IOP Conf. Ser. Mater. Sci. Eng. 202 012013
[7] Xingxing C, Xuebin Z, Hao S and Yi F 2014 Fabrication of Magnetic Fe3O4 Nanotubes by Electrospinning Rare Met. Mater. Eng. 43 2330–2334
[8] Frenot A and Chronakis I S 2003 Polymer nanofibers assembled by electrospinning Curr. Opin. Colloid Interface Sci. 8 64–75
[9] Bhardwaj N and Kundu S C 2010 Electrospinning: a fascinating fiber fabrication technique Biotechnol. Adv. 28 325–347
[10] Sill T J and von Recum H A 2008 Electrospinning: applications in drug delivery and tissue engineering Biomaterials 29 1989–2006
[11] Wahyudi T and Sugiyana D 2011 Pembuatan Serat Nano Menggunakan Metode Electrospinning Arena Tekst. 26
[12] Korbekandi H and Iravani S 2012 Silver nanoparticles The delivery of nanoparticles (InTech)
[13] Zhang Z-Q, Higgins J, Kim C L and Chang C 2013 Synthesis and antimicrobial properties of nanosilver J Mater Env. Sci 4 139–142
[14] Zhang Z, Wu Y, Wang Z, Zou X, Zhao Y and Sun L 2016 Fabrication of silver nanoparticles embedded into polyvinyl alcohol (Ag/PVA) composite nanofibrous films through electrospinning for antibacterial and surface-enhanced Raman scattering (SERS) activities Mater. Sci. Eng. C 69 462–469
[15] Tan G, Sağlam S, Emül E, Erdönmee D and Sağlam N 2016 Synthesis and characterization of silver nanoparticles integrated in polyvinyl alcohol nanofibers for bionanotechnological applications Turk. J. Biol. 40 643–651
[16] Lin S, Wang R-Z, Yi Y, Wang Z, Hao L-M, Wu J-H, Hu G-H and He H 2014 Facile and green fabrication of electrospin poly (vinyl alcohol) nanofibrous mats doped with narrowly dispersed silver nanoparticles Int. J. Nanomedicine 9 3937
[17] Cui W, Zhou Y and Chang J 2010 Electrospin nanofibrous materials for tissue engineering and drug delivery Sci. Technol. Adv. Mater. 11 014108
[18] Thompson C J, Chase G G, Yarin A L and Reneker D H 2007 Effects of parameters on nanofiber diameter determined from electrosprining model Polymer 48 6913–6922
[19] Kong H and Jang J 2008 Antibacterial Properties of Novel Poly(methyl methacrylate) Nanofiber Containing Silver Nanoparticles Langmur 24 2051–6
[20] Saquing C D, Manasco J L and Khan S A 2009 Electrospin nanoparticle–nanofiber composites via a one-step synthesis Small 5 944–951
[21] Ahamed M, Khan M M, Siddiqui M K J, AlSalhi M S and Alrokayan S A 2011 Green synthesis, characterization and evaluation of biocompatibility of silver nanoparticles Phys. E Low-Dimens. Syst. Nanostructures 43 1266–1271