Short Communication:
Synthesis of Colloidal Silver Nanoparticles in Various Liquid Media Using Pulse Laser Ablation Method and Its Antibacterial Properties

Syifa Avicenna, Iis Nurhasanah, and Ali Khumaeni*

Department of Physics, Faculty of Science and Mathematics, Diponegoro University,
Jl. Prof. Soedarto SH, Tembalang, Semarang 50275, Indonesia

*** Corresponding author:**

email: khumaeni@fisika.fsm.undip.ac.id

Received: October 4, 2020
Accepted: January 1, 2021

DOI: 10.22146/ijc.60344

Abstract: *The silver nanoparticles (AgNPs) have been applied as an antibacterial agent in consumer products, cosmetics, and food industries. In this present work, AgNPs were synthesized in various mediums of polyvinylpyrrolidone (PVP), polyethylene glycol (PEG), and chitosan using the pulse laser ablation synthesis method. Experimentally, a pulse Nd:YAG laser beam (1064 nm, 7 ns, 30 mJ) was directed using a silver mirror and focused using a quartz lens with a focal length of 30 mm on a silver metal plate placed in a petri dish containing liquid mediums for 120 min to produce colloidal silver nanoparticles. The results certified that All AgNPs have a spherical shape with polydisperse size in all media, including PVP, PEG, and chitosan. The smallest AgNPs have been produced in PVP medium with an averaged smallest size of 11.62 nm. Based on this result, PVP is the preferred medium to produce AgNPs with the smallest size and good stability. The produced silver nanoparticles have been successfully employed as an antibacterial agent, which is experimentally demonstrated by using Escherichia coli and Staphylococcus aureus. The result certified that the produced silver nanoparticles could effectively kill the bacteria with a killing percentage of 99.6 to 100%.*

Keywords: *silver nanoparticles; pulse laser ablation technique; Nd:YAG laser; liquid media of PVP; PEG; chitosan*

■ INTRODUCTION

Silver nanoparticles (AgNPs) have been well known as commercialized nano-material, having many beneficial applications. Due to their unique chemical and physical characteristics, including electrical, optical, thermal properties, the AgNPs have been employed in broad subjects including health care, medical, and industrial fields [1-3]. The AgNPs have been applied as an antibacterial agent in consumer products, cosmetics, medical tool coatings, and food industries [4-6]. In medical applications, the AgNPs have been used as anticancer agents in medical therapy, contrast agents in diagnostics, drug delivery, and agent of anticancer drugs. Thus, the synthesis of AgNPs has recently become an interesting subject.

Various techniques are suggested for the synthesis of AgNPs, such as sol-gel, precipitation, chemical reduction, ion sputtering techniques [7-8]. The techniques can successfully produce silver nanoparticles with quite good stability. However, the techniques involve high energy requirements and hazardous chemicals, which need further purification and are complicated in the synthesis process due to the fact the chemical contamination. Hence, the AgNPs cannot be employed readily for medical purposes or human-related products. Therefore, alternative techniques to produce high-purity AgNPs with good stability without involving dangerous chemical agents are necessary for specific applications in medical purposes and human-related products.

The other technique for the synthesis of AgNPs is the pulse laser ablation (PLA) technique [9]. In this technique, a pulse laser is used to ablate the metal material samples from their surface to induce a luminous plume. The plume then expands with time to disperse in a medium environment around the sample. When the medium is liquid, the ablated material disperses in the liquid medium, and finally, new material in the form of nanoparticles is produced. Compared to other chemical techniques, pulse laser ablation has several strong points. Namely, the produced nanoparticles have high purity because it does not involve chemical agents during the synthesis process and only needs pure metal and liquid medium such as deionized water. Furthermore, the pulse laser ablation technique has a much simple experimental setup than the case chemical technique. Many reports have been published on the synthesis of AgNPs using the PLA technique [10-15]. Tsuji et al. used the pulsed laser ablation technique to produce AgNPs in a water medium under the various wavelength of pulse laser, including 1064 nm, 532 nm, and 355 nm [15]. However, AgNPs cannot disperse well and is not quite stable because the particles will agglomerate and precipitate. To solve the problem, Bae et al. synthesized AgNPs by the laser pulse ablation method in NaCl solution, which resulted in an average nanoparticle size of 26.4 nm and very low stability compared to distilled water [10]. Then, in a dilute solution of sodium dodecyl sulfate (SDS) using the same method, the size of the AgNPs produced an average of 14 nm [13].

Valverde-Alva et al. used an ethanol medium to avoid agglomeration and precipitation [9]. Darroudi et al. employed gelatin solution to produce AgNPs having an average diameter ranging from 9 nm to 15 nm [12]. Al-Azawi et al. studied the effect of various liquid mediums, including deionized water, ethanol, and polyvinylpyrrolidone (PVP), on the particle size, resulting in the finer particle size for PVP liquid medium compared to the case of deionized water and ethanol [16]. It should be mentioned that the above reports are mainly used high-energy pulse laser, namely more than 50 mJ. Furthermore, they only studied the synthesis of silver nanoparticles and their characterization, including pulse laser energy dependence and liquid medium dependence. They did not report simultaneously the application of the produced silver nanoparticles for an antibacterial agent.

In this present work, we conducted a study on the synthesis of AgNPs in various mediums of polyvinylpyrrolidone (PVP), polyethylene glycol (PEG), and chitosan using the pulse laser ablation synthesis method. The laser energy used was only 30 mJ. As mentioned above, the use of the pulsed laser ablation technique for the preparation of AgNPs in this study is because this technique can produce high-purity nanoparticles without additional hazardous chemical agents for medical applications and human-related products. The liquid medium has a role in regulating the size of a nanoparticle, where the addition of a polymer medium can produce a smaller and more uniform nanoparticle size. The effect of the liquid medium on the averaged sizes of produced AgNPs was studied. Characterization of AGNPs was carried out using the Ultraviolet-Visible Spectroscopy (UV-Vis) method, Particle Size Analyzer (PSA), Transmission Electron Microscope (TEM), and Fourier Transform Infrared spectroscopy (FTIR). Furthermore, produced silver nanoparticles are then applied as antibacterial agents, which are experimentally demonstrated by using Escherichia coli and Staphylococcus aureus. The result certified that the produced silver nanoparticles could effectively kill the bacteria with a killing percentage of 99.6 to 100%.

■ EXPERIMENTAL SECTION

Materials

A silver metal plate with a purity of 99.9% and a dimension of $5 \times 10 \times 20$ mm^3 was used as a material target to produce AgNPs. The liquid medium for making colloidal AgNPs used were PVP, PEG, and Chitosan.

Instrumentation

The experimental setup used in this work is shown in Fig. 1. The radiation source used was Nd:YAG laser (New Wave Research, Polaris II, 20 Hz) with a wavelength of 1064 nm and pulse width of 7 ns. Laser Exec II software was used to set laser parameters (energy, repetition rate). The laser energy was set at 30 mJ with a repetition rate of 10 Hz. The other instruments used in this work include a transmission electron microscope

(TEM, JEOL) equipped with an energy dispersive X-ray spectrometer (EDX) and ultraviolet-visible (UV-Vis) absorption spectrometer (Shimadzu 1240 SA).

Procedure

Synthesis of silver nanoparticles

Experimentally, a pulse laser beam was directed using a silver mirror and focused using a quartz lens with a focal length of 30 mm on a silver metal plate placed in a petri dish containing liquid mediums for 11 h. With increasing the number of laser bombardment, the color of the liquid medium changes from transparent to light yellow and finally become brownies yellow. The experimental setup used in this work is shown in Fig. 1.

Characterization of silver nanoparticles

The produced colloidal AgNPs were characterized using various techniques, including transmission electron microscope (TEM), particle size analyzer (PSA), ultraviolet-visible (UV-Vis) spectroscopy, and Fourier Transform Infra-red (FTIR) spectroscopy to obtain a morphological view, average size, optical plasmon resonance, and molecular ingredient of colloidal AgNPs, respectively. The morphology of the nanoparticles produced was analyzed using a transmission electron microscope (TEM, JEOL) equipped with an energy dispersive X-ray spectrometer (EDX). The optical properties and surface plasma resonance of the product were characterized by an ultraviolet-visible (UV-Vis) absorption spectrometer (Shimadzu 1240 SA).

Antibacterial testing

The antibacterial test was made using AOAC 960.09 the year 2013 with slight modification [17]. The bacteria used were *Escherichia coli* (ATCC 8739) and *Staphylococcus aureus* (ATCC 6538). The temperature of incubation was 35 °C, and the temperature of the examination was 24 °C. A sample of 10 ppm AgNPs was inoculated with microorganisms tests including *E. coli* and *S. aureus* with a concentration of 1.3×10^6 CFU/mL and 1.1×10^6 CFU/mL, respectively, at a contact time of 10 min. One mL of AgNPs was poured with 1 m: of test suspension. This mixture was then striated in the solid medium of TSA in four quadrants with 4 striae each on the surface of the agar. Each quadrant was incubated for 48 h at 35 °C. After incubation, the growth of the inoculated microorganism in each quadrant was counted.

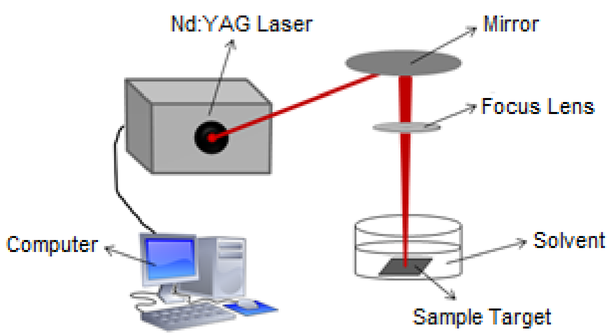

Fig 1. Experimental setup

■ RESULTS AND DISCUSSION

Effect of Liquid Medium on Silver Nanoparticles (AgNPs)

First, we examined the physical characteristics of colloidal silver nanoparticles produced in various liquid media. Fig. 2 shows photographs of colloidal AgNPs produced in (a) PEG, (b) PVP, and (c) chitosan using the pulse laser ablation method. For producing these colloidal AgNPs, the Nd:YAG laser beam with a pulse repetition rate of 10 Hz was bombarded and focused on a high-purity Ag metal plate for 11 h. It can be seen in the figure that the formation of colloidal AgNPs in the three liquid mediums is identified by the color changing of the liquid medium that was previously colorless or clear to golden yellow. The golden yellow color of colloidal silver nanoparticles indicates the formation of silver nanoparticles as reported elsewhere [18].

The darker color of the colloid indicates a higher concentration of AgNPs [19]. The distinctive color of AgNPs is the result of the localized surface plasmon

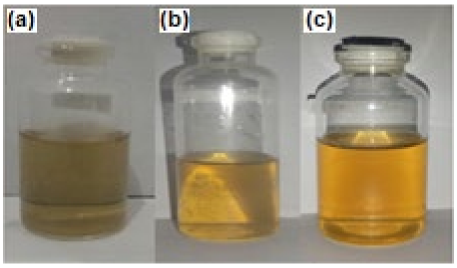

Fig 2. Colloidal AgNPs synthesized by pulsed laser ablation technique in liquid medium (a) PEG, (b) PVP, and (c) Chitosan

resonance (LSPR) phenomenon where silver material absorbs. The LSPR of AgNPs ranges from 380 to 425 nm, which means that they absorb blue or red light, so it will reflect the yellow color [18,20-22]. For further information, it will be discussed in the characterization section.

The colloidal AgNPs were subsequently stored in transparent bottles at room temperature for several weeks. The results showed that AgNPs were the most stable in the chitosan medium because they managed to survive without precipitation for 6 weeks, whereas in the PEG medium, nanoparticles were stable for 5 weeks. Also, in the PVP medium, the nanoparticles lasted for 4 weeks until black sedimentation appeared at the bottom of the bottle.

Analysis of Morphology and Size of Silver Nanoparticles

To determine the morphology and size of the nanoparticles, measurements were made using a transmission electron microscope (TEM) and a particle size analyzer (PSA), respectively. The PSA uses a dynamic light scattering (DLS) principle that takes advantage of the properties of particles, which experience Brownian motion. Brownian motion of the liquid particles collides with each other so that when a laser beam is fired, it will cause scattering of light with various frequencies. This frequency will later be converted into a light signal form, which is then converted again into a digital signal, which is then processed into a counting series [23]. Fig. 3 shows the histogram of the size distribution and morphology of AgNPs in liquid media of PVP, PEG, and chitosan. Fig. 3(a) shows the size distribution of AgNPs in PVP, which consists of two peaks. For the large group average size, which is above 100 nm, it has an average particle size of 275.80 ± 38.96 nm, then for the small size group, it is 11.62 ± 5.03 nm. Fig. 3(b) shows a histogram of the size distribution of AgNPs in PEG, which also consists of two peaks. Namely, the peaks of the large particle groups had an average particle size of 146.60 ± 28.02 nm and the peaks of the small particle groups had an average particle size of 21.02 ± 3.38 nm. Then, Fig. 3(c) shows the average histogram size distribution of AgNPs in chitosan liquid medium, which is also divided into two peaks, that is a large particle group with an average particle size of 480.60 ± 163.40 nm and a small particle group with an average particle size of 71.60 ± 19.43 nm.

From the size distribution data above, it is known that the colloidal AgNPs formed to have two nanoparticle size distributions. This certified that the AgNPs colloids consist of polydisperse colloids composed of more than one peak or have different sizes that are quite far apart from each other [24]. The non-uniform size of the nanoparticles produced in each sample is due to the Brownian motion that occurs due to the pulse laser ablation method, as also reported in a paper here [25]. In addition, the type of colloid medium affects the uniformity of the size of the produced AgNPs. PVP polymers have a smaller aggregate ability than others, resulting in more small particle sizes. If the particles are agglomerated, they will produce large size nanoparticles [26], such as in PEG and chitosan, which have a size larger than PVP. The results of the size distribution of the

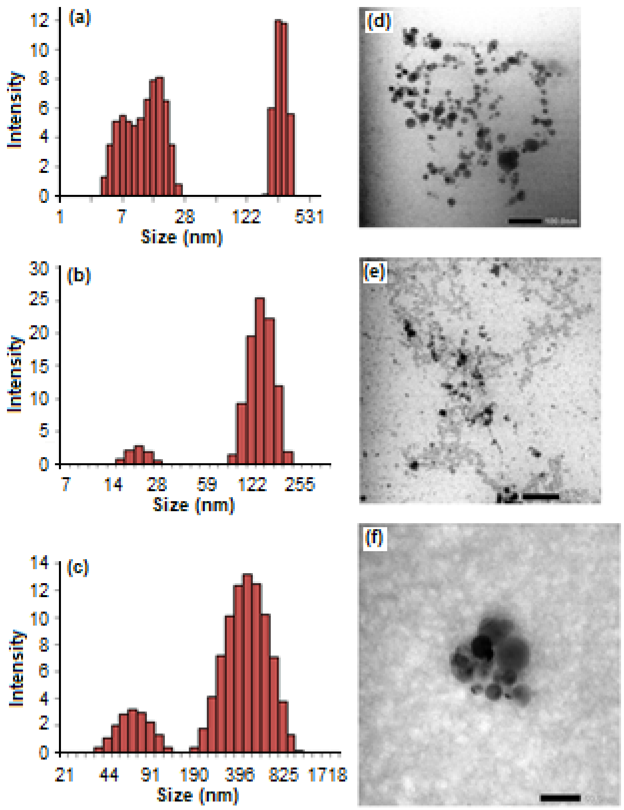

Fig 3. Histograms of the size distribution of AgNPs in (a) PVP, (b) PEG, (c) chitosan, and AgNPs morphology in (d) PVP, (e) PEG, and (f) chitosan using PSA and TEM

three colloids indicate that AgNPs have been successfully synthesized according to the literature, which is between 1–1000 nm in size [26-27].

Optical Properties of AgNPs Using UV-Vis Spectrometer

To study the optical characteristics of produced AgNPs, the UV-Vis spectrometer was used. Based on the image shown in Fig. 4, all three graphs have absorbance peaks around the 400 nm wavelength. This peak is defined as the peak of localized surface plasmon resonance. Light single Plasmon resonance (LSPR) is an important parameter to identify the type of particles contained in a solution. LSPR is a resonance phenomenon between light waves and electrons on the metal surface, which oscillate with each other. LSPR occurs when the frequency of the photons matches the collective oscillations of the metal nanoparticles. The frequency and intensity of LSPR absorption depend on the type of material (metal), size, shape of the nanostructures, and the environment [28].

LSPR occurs when the frequency of the photons matches the collective oscillations of the metal nanoparticles. The frequency and intensity of LSPR absorption depend on the type of material (metal), size, shape of the nanostructures, and the environment [21]. The LSPR peak of silver nanoparticles in the PVP medium is at a wavelength of 408 nm with an absorbance of 1.118. Then, in the PEG medium, the peak is at a wavelength of 422 nm with an absorbance of 0.648. Whereas, in the chitosan medium, the SPR was at a wavelength of 408 nm with an absorbance of 1.012. According to literature, silver has an LSPR peak of around 380–425 nm [21-22]. The peak shift of 14 nm, as shown in the PEG graph, shows a larger size of the nanoparticles. Also, the absorbance amount can also indicate the size because the larger the nanoparticle size also provides a high absorbance. This is because the higher the absorbance usually indicates a higher concentration. High concentrations tend to give larger nanoparticles size [29].

Compound Composition of AgNPs Colloidal

Fourier transform infrared (FTIR) was used to determine the compounds contained in colloids. The colloidal infrared transmittance spectrum of AgNPs can be seen in Fig. 5. It shows a graph of the wavenumber (cm^{-1}) against the percent transmittance passing through the material (% T). The absorption band of AgNPs at a wavenumber of 3359 cm^{-1} showed a vibration of O–H stretching with a transmittance of 86%.

Then, the wavenumber 1739 cm^{-1} indicates the presence of a carbonyl group C=O with a transmittance of 92%. The absorption bands at wavenumbers 3359 cm^{-1} and 1739 cm^{-1} indicate the presence of other molecules absorbed from the chitosan medium associated with AgNPs. The wavenumber of 528 cm^{-1} shows a stretching vibration of the Ag–O bond with a transmittance of 80%. This is evidence of the existence of AgO and Ag_2O [30].

Application of AgNPs as an Antibacterial Agent

Finally, the application of AgNPs was demonstrated as an antibacterial agent. To this end, colloidal AgNPs produced in PVP medium with a concentration of 10 mg/kg AgNPs were examined as an

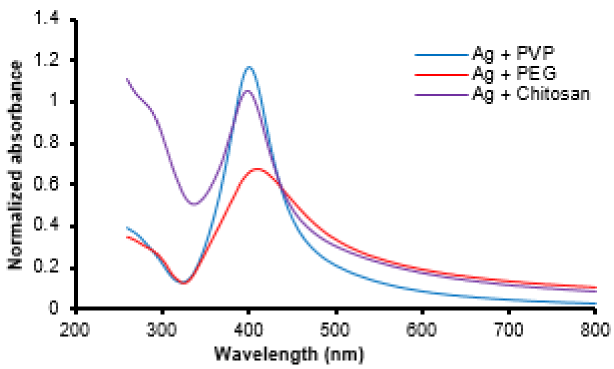

Fig 4. UV-Vis spectrum of AgNPs colloid in PVP, PEG, and Chitosan liquid medium

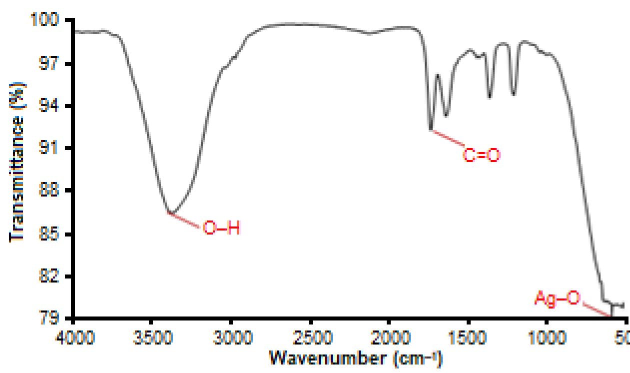

Fig 5. IR transmittance spectrum of colloidal AgNPs on chitosan medium

Table 1. AgNPs as an antibacterial agent in gram-negative *Escherichia coli* and gram-positive *Staphylococcus aureus*

Contamination time (sec)	Number of initial bacteria (CFU/mL)	Number of remaining bacteria (CFU/mL)	Number of dead bacteria (%)
Escherichia coli			
30	1.3×10^6	0	100
60	1.3×10^6	0	100
Staphylococcus aureus			
30	1.1×10^6	3.9×10^3	99.64
60	1.1×10^6	1.1×10^2	99.99

antibacterial agent. The produced AgNPs used in this antibacterial agent has a spherical shape with an average small diameter of 11.62 nm.

Table 1 shows the examination results of AgNPs as an antibacterial agent in gram-negative Escherichia coli and gram-positive Staphylococcus aureus. For this experiment, the number of initial bacteria is 1.3×10^6 CFU/mL and 1.1×10^6 CFU/mL, respectively. It can be seen that after 30 sec of AgNPs treatment to *E. coli* bacteria, 100% of bacteria are dead, and completely no bacteria remain. For *S. aureus*, 99.64% of *S. aureus* bacteria are dead. The dead bacteria increased up to 99.99% when the interaction time of bacteria with AgNPs was much longer, up to 60 sec. This result certified that the produced colloidal AgNPs in PVP medium with a concentration of 10 mg/kg could effectively be used as an antibacterial agent. As reported here [31], the interaction mechanism between AgNPs and bacteria is as follows: the AgNPs attach to the surface of the cell wall and membrane. After that, the AgNPs penetrates inside the cell and inducing cellular toxicity and oxidative stress by generating reactive oxygen species and free radicals, damaging intracellular structures, and biomolecules.

■ CONCLUSION

We demonstrated in this work that colloidal nanosilver particles have been successfully produced by using the pulse laser ablation method in various liquid media, including PVP, PEG, and chitosan. In this work, a pulse Nd:YAG laser with a laser wavelength of 1064 nm, laser energy of 30 mJ, and a pulse repetition rate of 10 Hz was employed as an energy source. The results certified that All AgNPs have a spherical shape with polydisperse size in all media, including PVP, PEG, and chitosan. The smallest AgNPs have been produced in PVP medium with an average smallest size of 11.62 nm. Furthermore, AgO compounds were also produced during the synthesis process. Based on this result, PVP is the preferred medium to produce AgNPs with the smallest size and good stability. In this present work, AgNPs have been successfully applied as antibacterial agents in *Escherichia coli* and *Staphylococcus aureus* bacteria.

■ ACKNOWLEDGMENTS

This work has been financially supported by Research Grant from the Ministry of Education and Culture, Indonesia, under the research contract of PTUPT with a contract No. 225-135/UN7.6.1/PP/2020.

■ REFERENCES

[1] Kruszewski, M., Brzoska, K., Brunborg, G., Asare, N., Dobrzyńska, M., Dušinská, M., Fjellsbø, L.M., Georgantzopoulou, A., Gromadzka-Ostrowska, J., Gutleb, A.C., Lankoff, A., Magdolenová, Z., Pran, E.R., Rinna, A., Instanes, C.J., Sandberg, W., Schwarze, P., Stępkowski, T., Wojewódzka, M., and Refsnes, M., 2011, Toxicity of silver nanomaterials in higher eukaryotes, *Adv. Mol. Toxicol.*, 5, 179–218.

[2] Marassi, V., Di Cristo, L., Smith, S.G.J., Ortelli, S., Blosi, M., Costa, A.L., Reschiglian, P., Volkov, Y., and Prina-Mello, A, 2018, Silver nanoparticles as a medical device in healthcare settings: A five-step approach for candidate screening of coating agents, *R. Soc. Open Sci.*, 5 (1), 171113.

[3] Jing, X., and Guo, Z., 2019, Multifunctional WS_2&M-AgNPs superhydrophobic conductive sponges for application in various sensors, *New J. Chem.*, 43 (4), 5287–5296.

[4] Natsuki, J., Natsuki, T., and Hashimoto, Y., 2015, A review of silver nanoparticles: Synthesis methods, properties and applications, *Int. J. Mater. Sci. Appl.*, 4 (5), 325–332.

[5] Carbone, M., Donia, D.T., Sabbatella, G., and Antiochia, R., 2016, Silver nanoparticles in polymeric matrices for fresh food packaging, *J. King Saud Univ. Sci.*, 28 (4), 273–279.

[6] Divya, M., Kiran, G.S., Hassan, S., and Selvin, J., 2019, Biogenic synthesis and effect of silver nanoparticles (AgNPs) to combat catheter-related urinity tract infections, *Biocatal. Agric. Biotechnol.*, 18, 101037.

[7] Lkhagvajav, N., Yaşa, I., Çelik, E., Koizhaiganova, M., and Sari, Ö., 2011, Antimicrobial activity of colloidal silver nanoparticles prepared by sol-gel method, *Dig. J. Nanomater. Biostruct.*, 6 (1), 149–154.

[8] Mishra, Y.K., Mohapatra, S., Kabiraj, D., Mohanta, B., Lalla, N.P., Pivin, J.C., and Avasthi, D.K., 2007, Synthesis and characterization of Ag nanoparticles in silica matrix by atom beam sputtering, *Scr. Mater.*, 56 (7), 629–632.

[9] Valverde-Alva, M.A., García-Fernández, T., Villagrán-Muniz, M., Sánchez-Aké, C., Castañeda-Guzmán, R., Esparza-Alegría, E., Sánchez-Valdés, C.F., Sánchez Llamazares, J.L., and Herrera, C.E.M., 2015, Synthesis of silver nanoparticles by laser ablation in ethanol: A pulsed photoacoustic study, *Appl. Surf. Sci.*, 355, 341–349.

[10] Bae, C.H., Nam, S.H., and Park, S.M., 2002, Formation of silver nanoparticles by laser ablation of a silver target in NaCl solution, *Appl. Surf. Sci.*, 197-198, 628–634.

[11] Boutinguiza, M., Comesaña, R., Lusquiños, F., Riveiro, A., del Val, J., and Pou, J., 2015, Production of silver nanoparticles by laser ablation in open air, *Appl. Surf. Sci.*, 336, 108–111.

[12] Darroudi, M., Ahmad, M.B., Zamiri, R., Abdullah, A.H., Ibrahim, N.A., Shameli, K., and Shahril Husin, M., 2011, Preparation and characterization of gelatin mediated silver nanoparticles by laser ablation, *J. Alloys Compd.*, 509 (4), 1301–1304.

[13] Mafune, F., Kohno, J., Takeda, Y., and Kondow, T., 2000, Formation and size control of silver nanoparticles by laser ablation in aqueous solution, *J. Phys. Chem. B*, 104 (39), 9111–9117.

[14] Rhim, J.W., Wang, L.F., Lee, Y., and Hong, S.I., 2014, Preparation and characterization of bionanocomposite films of agar and silver nanoparticles: Laser ablation method, *Carbohydr. Polym.*, 103, 456–465.

[15] Al-Azawi, M.A., Bidin, N., Bououdina, M., Abbas, K.N., Al-Asedy, H.J., Ahmed, O.H., and Thahe, A.A., 2016, The effects of the ambient liquid medium on the ablation efficiency, size and stability of silver nanoparticles prepared by pulse laser ablation in liquid technique, *J. Teknol.*, 78 (3), 7–11.

[16] Song, X., Vossebein, L., and Zille, A., 2019, Efficacy of disinfectant-impregnated wipes used for surface disinfection in hospitals: A review, *Antimicrob. Resist. Infect. Control*, 8 (1), 139.

[17] Mulfinger, L., Solomon, S.D., Bahadory, M., Jeyarajasingam, A.V., Rutkowsky, S.A., and Boritz, C., 2007, Synthesis and study of silver nanoparticles, *J. Chem. Educ.*, 84 (2), 322–325.

[18] Ahmed, B.S., Rao, A.G., Sankarshan, B.M., Vicas, C.S., Namratha, K., Umesh, T.K., Somashekar, R., and Byrappa, K., 2016, Evaluation of gold, silver, and silver-gold (bimetallic) nanoparticles as radiosensitizers for radiation therapy in cancer treatment, *Cancer Oncol. Res.*, 4 (3), 42–51.

[19] Abadeer, N.S., and Murphy, C.J., 2016, Recent progress in cancer thermal therapy using gold nanoparticles, *J. Phys. Chem. C*, 120 (9), 4691–4716.

[20] Evanoff, D.D., and Chumanov, G., 2005, Synthesis and optical properties of silver nanoparticles and arrays, *ChemPhysChem*, 6 (7), 1221–1231.

[21] Rodríguez-León, E., Iñiguez-Palomares, R., Navarro, R.E., Herrera-Urbina, R., Tánori, J., Iñiguez-Palomares, C., and Maldonado, A., 2013, Synthesis of silver nanoparticles using reducing agents obtained from natural sources (*Rumex hymenosepalus* extracts), *Nanoscale Res. Lett.*, 8 (1), 318.

[22] Nuraeni, W., Daruwati, I., Widyasari, E.M., and Sriyani, M.E., 2013, Verifikasi kinerja alat particle size analyzer (PSA) HORIBA LB-550 untuk

penentuan distribusi ukuran nanopartikel, *Prosiding Seminar Nasional Sains dan Teknologi Nuklir*, PTNBR-BATAN, Bandung, 4 July 2013, 266–271.
[23] Lichtenegger, T., and Pirker, S., 2018, CFD-DEM modeling of strongly polydisperse particulate systems, *Powder Technol.*, 325, 698–711.
[24] Kim, M., Osone, S., Kim, T., Higashi, H., and Seto, T., 2017, Synthesis of nanoparticles by laser ablation: A review, *KONA Powder Part. J.*, 34, 80–90.
[25] Tejamaya, M., Merrifield, R.C., and Lead, J.R., 2012, Stability of citrate, PVP, and PEG coated silver nanoparticles in ecotoxicology media, *Environ. Sci. Technol.*, 46 (13), 7011–7017.
[26] Tiyaboonchai, W., 2003, Chitosan nanoparticles: A promising system for drug delivery, *Naresuan Univ. J.*, 11 (3), 51–66.
[27] Beyene, H.D., Werkneh, A.A., Bezabh, H.K., and Ambaye, T.G., 2017, Synthesis paradigm and applications of silver nanoparticles (AgNPs), a review, *Sustainable Mater. Technol.*, 13, 18–23.
[28] Chhatre, A., Solasa, P., Sakle, S., Thaokar, R., and Mehra, A., 2012, Color and surface plasmon effects in nanoparticle systems: Case of silver nanoparticles prepared by microemulsion route, *Colloids Surf., A*, 404, 83–92.
[29] Hosseinpour-Mashkani, S.M., and Ramezani, M., 2014, Silver and silver oxide nanoparticles: Synthesis and characterization by thermal decomposition, *Mater. Lett.*, 130, 259–262.
[30] Dakal, T.C., Kumar, A., Majumdar, R.S., and Yadav, V., 2016, Mechanistic basis of antimicrobial actions of silver nanoparticles, *Front. Microbiol.*, 7, 1831.