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Indonesian Journal of Chemistry

1. Draft manuskrip sebelum proses submission
2. Submission acknowledgment dari Indonesian Journal of Chemistry (4 Oktober 2020)
3. Editor and reviewers' comments (22 November 2020)
4. Balasan komentar Editor dan Reviewers
5. Paper setelah proses revisi mempertimbangkan masukan Editor dan Reviewers
6. Mengirim komentar dan revisi manuskrip ke Jurnal (9 Desember 2020)
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1. **Draft manuskrip sebelum proses submission**

Synthesis of colloidal silver Nanoparticles in various liquid media using pulse laser ablation method and its antibacterial properties

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Abstract: *The silver nanoparticles (AgNPs) have been applied as an antibacterial agent in consumer products, cosmetics, medical tool coatings, and food industries. The technique generally used for synthesis of AgNPs is chemical reduction. However, the techniques involves high energy requirements and hazardous chemicals, which need further purification and are complicated in synthesis process due to the fact the chemical contamination. In this present work, AgNPs were synthesized in various medium of polyvinylpyrrolidone (PVP), polyethylene glycol (PEG), and chitosan using the pulse laser ablation synthesis method.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. Effect of liquid medium to the averaged sizes of produced AgNPs were 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). The results show that the AgNPs produced in PVP liquid media have smallest size compared to other media of PEG and chitosan.*

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

INTRODUCTION

Silver nanoparticles (AgNPs) has 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 broaden 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 deliver, agent of anticancer drugs. Thus, synthesis of AgNPs has recently become an interest 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 a quiet good stability. However, the techniques involves high energy requirements and hazardous chemicals, which need further purification and are complicated in synthesis process due to the fact the chemical contamination. Hence, the AgNPs cannot be employed readily for medical purpose 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 purpose and human-related products.

The other technique is usually applied to synthesis of AgNPs is pulse laser ablation (PLA) technique [9–10]. 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 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 technique, pulse laser ablation has several strong points. Namely, the produced nanoparticles has high purity because it does not involve chemical agents during synthesis process and only needs pure metal and liquid medium such as deionized water. Furthermore, the pulse laser ablation technique has much simple experimental setup than the case of chemical technique. Many reports on synthesis of AgNPs using PLA technique have been published as elsewhere [9–10] . Tsuji et al. used pulse laser ablation technique to produce AgNPs in water medium under various wavelength of pulse laser including 1064 nm, 532 nm, and 355 nm. However, AgNPs cannot disperse well and is not quit stable because the particles will agglomerate and precipitate. In previous studies, AgNPs were synthesized 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. 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.

Valverde-Alva et al. used ethanol medium to avoid agglomeration and precipitation. Darroudi et al. employed gelatin solution to produce AgNPs having average diameter ranging from 9 nm to 15 nm. Al-Azawi et al. studied the effect of various liquid medium including deionized water, ethanol, and polyvinilpirrolidone (PVP) to the particle size, resulting in the finer particle size for PVP liquid medium compared to the case of deionized water and ethanol [10].

In this present work, AgNPs were synthesized in various medium of polyvinylpyrrolidone (PVP), polyethylene glycol (PEG), and chitosan using the pulse laser ablation synthesis method.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. Effect of liquid medium to the averaged sizes of produced AgNPs were 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).

EXPERIMENTAL SECTION

Materials

Silver metal plate with purity of 99,9 % and a dimension of 5x 10 x 20 mm3 was used as a material target to produce AgNPs. Liquid medium for making colloidal AgNPs used were PVP, PEG, and Chitosan.

Instrumentation

The experimental set up 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 wavelengths 1064 nm and pulse widths 7 ns. Laser Exec II software was used to set laser parameters (energy, repetition rate). The laser energy was set at 45 mJ with a repetition rate of 10 Hz.

Procedure

Synthesis of tin oxide nanoparticles

Experimentally, a pulse laser beam was directed using silver mirror and focused using quartz lens with a focal length of 30 mm on a silver metal plate placed in a petri dish containing liquid mediums for 120 minutes. With increasing a number of laser bombardment, the color of liquid medium changes from transparent to light yellow and finally become brownies yellow. The experimental set up used in this work is shown in Fig. 1.

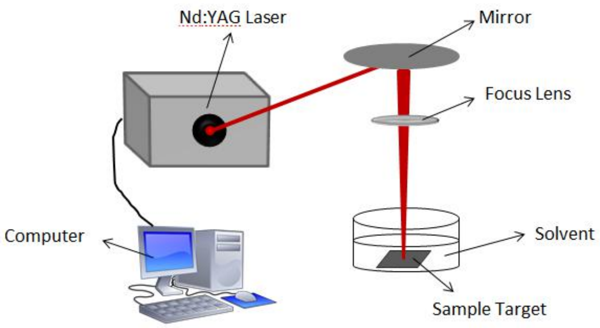


Fig. 1. Experimental setup

Characterization of tin oxide nanoparticles

The produced colloidal AgNPs were the characterized using various techniques including

TEM, PSA, UV-Vis, and FTIR to obtain 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).

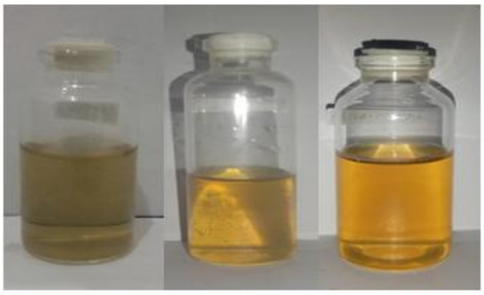
Antibacterial testing

The antibacterial test was made using AOAC 960.09 year 2013. The bacteria used were *Escherichia coli* (ATCC 8739) and *Staphylococcus aureus* (ATCC 6538). The temperature of incubation was 35°C and the temperature of examination was 24°C.

■ RESULTS AND DISCUSSION

Effect of liquid medium on silver nanoparticles (AgNPs)

Synthesis results in aquadest and 5 M EG media First, we examined the physical characteristics of colloidal silver nanoparticles produced in various liquid media. Figure 2 shows photographs of colloidal AgNPs produced in (a) PEG, (b) PVP, and (c) chitosan using 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 high-purity Ag metal plate for 11 hours. It can be seen in the figure that the formation of colloidal AgNPs in the three liquid mediums is identified by 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 in elsewhere [11].



(a) (b) (c)

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

The darker color of the colloid indicates a higher AgNPs [12]. The distinctive color of AgNPs is the result of the localized surface plasmon resonance (LSPR) phenomenon where silver material absorb. 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 [11], [13]–[15]. 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), repesctively. 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 [16]. Figure 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 consist 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

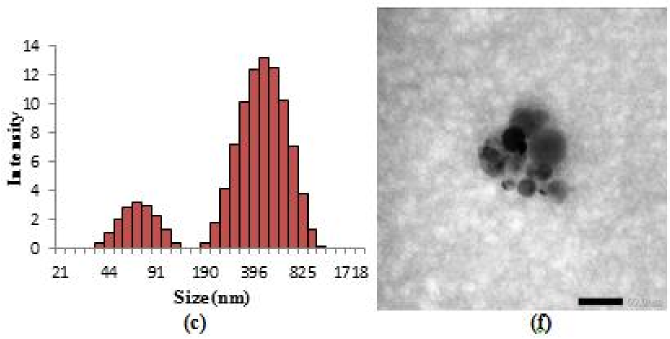
± 5.03 nm. Figure 3(b) shows a histogram of the size distribution of AgNPs in PEG, which also consists of two peaks. Namely, the peaks of the large particle groups had an average particle size of 146.60 ± 28.02 nm and the peaks of the small particle groups had an average particle size of 21.02 ± 3.38 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.

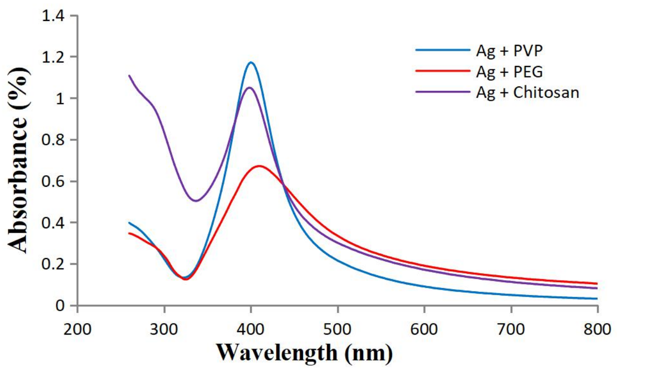
From the size distribution data below, it is known that the colloidal AgNPs formed has two nanoparticle size distributions. This certified that the AgNPs colloids consists of polydisperse colloids that are composed of more than one peak or have different sizes that are quite far apart from each other [17]. 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 [18]. In addition, the type of colloid medium also 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, it will produce large size nanoparticles [19], such as in PEG and chitosan which have a size larger than PVP. The results of the size distribution of the three colloids indicate that AgNPs have been successfully synthesized according to the literature, which is between 1-1000 nm in size [19–20].

Fig. 3. Histograms of 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

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 oscillates 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, the shape of the nanostructures, and the environment [21].





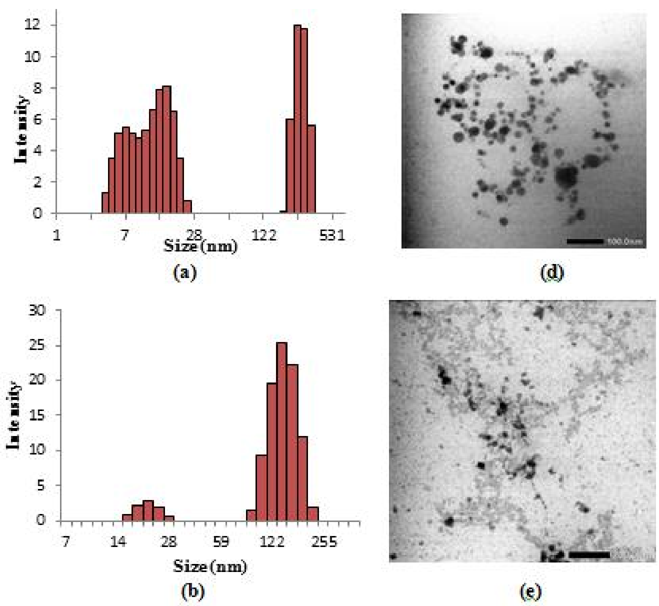


Fig. 4. UV-Vis spectrum of AgNPs colloid in PVP, PEG, and Chitosan liquid medium

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, the 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 a.u. Then, in the PEG medium, the peak is at a wavelength of 422 nm with an absorbance of 0,648 a.u. whereas, in the chitosan medium, the SPR was at a wavelength of 408 nm with an absorbance of 1,012 a.u. According to literature, silver has an LSPR peak of around 380 - 425 nm [14–15]. The peak shift of 14 nm as shown in the PEG graph shows a larger size of the nanoparticles. In addition, 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 the higher the concentration. High concentrations tend to give larger nanoparticles size [22].

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

Contamination time (s)	Number of initial bacteria (CFU/ml)	Number of remaining bacteria (CFU/ml)	Number of dead bacteria (%)
30	1.1 x 10^6	3.9 x 10^3	99.64
60	1.1 x 10^6	1.1 x 10^2	99.99

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 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 Ag2O [23].

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

Application of AgNPs as an antibacterial agent

Finally, application of AgNPs was demonstrated as

Contamination time (s)	Number of initial bacteria (CFU/ml)	Number of remaining bacteria (CFU/ml)	Number of dead bacteria (%)
30	1.3 x 10^6	0	100
60	1.3 x 10^6	0	100

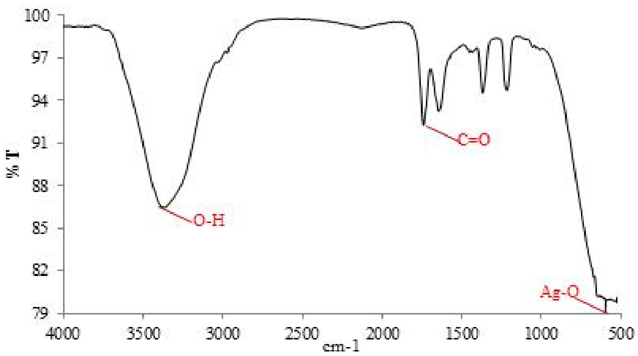
an antibacterial agent. To this end, colloidal AgNPs produced in PVP medium with a concentration of 10 mg/kg AgNPs were examined as antibacterial agent.

Table 1. AgNPs as antibacterial agent in gram-negative Escherichia coli

Table 1 shows the examination results of AgNPs as antibacterial agent in gram-negative Escherichia coli. For this experiment, number of initial bacteria is 1.3 x 10^6 CFU/ml. It can clearly be seen that after 30 s of AgNPs treatment to E coli bacteria, 100 % bacteria is dead and completely no bateria remain. This result certified that the produced colloidal AgNPs in PVP medium with a concentration of 10 mg/kg can effectively be used as an antibacterial agent.

Table 2. AgNPs as antibacterial agent in gram-positive Staphyloccus aureus

Further examination was also made in gram-positive Staphylococcus aureus. The results show



that after 30 s interaction of bacteria with 10 mg/kg AgNPs, 99.64 % S aeureus bacteria is dead. The dead bacteria increased up to 99.99 % when the interaction time of bacteria with AgNPs was much longer up to 60 s. This results confirmed that the AgNPs can also be employed as antibacterial agent in S aureus.

CONCLUSION

We demonstrated in this work that colloidal nanosilver particles have been successfully produced by using 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 45 mJ, and pulse repetition rate of 10 Hz was employed as an energy source. The results certified that All AgNPs have spherical shape with polidisperse size in all media including PVP, PEG, and chitosan. The smallest AgNPs have been produced in PVP medium with averaged smallest size of 11.62 nm. Furthermore, AgO compound also produced during synthesis process. Based on this result, PVP is preferable medium to produce AgNPs with smallest size and good stability. In this present work, AgNPs have been succesfully applied as antibacterial agents in E coli and S aureus bacteria.

ACKNOWLEDGMENTS

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

REFERENCES

[1] Kruszewski, M.,et al., 2011,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., Prina-Mello, A., 2018, R. Soc. Open Sci., 5(2)
[3] Chen, J. C., Lin, Z. H., Ma, X. X., 2003, Lett. Appl. Microbiol., 37(2), 105–108
[4] Natsuki, J., Natsuki, T., Hashimoto, Y., 2015, Int. J. Mater. Sci. Appl., 4(5), 325–332
[5] Bosetti, M., Massè, A., Tobin, E., Cannas, M.,(2002),Biomaterials, 23(3), 887–892
[6] Yeo, S.Y., Lee, H.J., Jeong, S.H., 2003, J. Mater. Sci., 38(10), 2143–2147
[7] Lkhagvajav, N., Ya, I Ş A., Çel, E İ K., Koizhaiganova, M., Sari, Ö., 2011,Journal of Nanomaterials and Biostructures, 6(1), 149–154
[8] Mishra, Y.K,et al., 2007, Scripta Materialia, 56, 629–632
[9] Bae, C.H., Nam, S.H., and Park, S.M., 2002,Appl. Surf. Sci., 197–198, 628–634
[10] Valverde-alva, M A, et al., Applied Surface Science,355, 341–349
[11] Solomon, Sally D., et al., (2007), J. Chem. Educ.,84(2), 322–325
[12] Ahmed, B.S,et al., (2016), Cancer Oncol. Res., 4(3) 42–51
[13] Abadeer, Nardine S., Murphy, Catherine J., 2016, . Phys. Chem. C, 120(9),4691
[14] Evanoff Jr D.D and Chumanov G., Chem.Phys.Chem, pp. 1221–1231, 2005,
[15] Rodríguez-León,E. et al., Nanoscale Res. Lett., vol. 8, no. 1, pp. 1–9, 2013
[16] Nuraeni,W., Daruwati, I., E. M. W, and Sriyani,M. E., 2013, PTNBR- BATAN Bandung, 266–271
[17] Lichtenegger,T. and Pirker,S., 2018, Powder Technol., 325, 698–711
[18] Kim,S., Osone,M., Kim,T., Higashi,H., and Seto,T., 2017, KONA Powder Part. J., no. 34, 80–90
[19] Tejamaya,M., Merrifield, R. C., and Lead,J. R., 2012, Environmental Science and Technology.
[20] Tiyaboonchai,W., 2003, Naresuan Univ. J., 11(3), 51–66
[21] Minh Hiep,H., et al., Sci.,2007, Technol. Adv. Mater., 8(4), 331–338
[22] Chhatre,A., Solasa,P., Sakle,S., Thaokar,R., and Mehra,A., 2012, Colloids Surfaces A Physicochem. Eng. Asp., 404, 83–92,
[23] Hosseinpour-Mashkani,S. M. and Ramezani M., 2014, Mater. Lett., vol. 130, pp. 259–262

2. **Submission acknowledgment dari Indonesian Journal of Chemistry (4 Oktober 2020)**

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[IJC] Submission Acknowledgement

1 message

Prof. Dr.rer.nat. Nuryono, MS <nuryono_mipa@ugm.ac.id> Sun, Oct 4, 2020 at 1:01 PM
To: Dr Ali Khumaeni <khumaeni@fisika.fsm.undip.ac.id>

Dear Dr Ali Khumaeni,

Thank you for submitting the manuscript, "Synthesis of colloidal silver Nanoparticles in various liquid media using pulse laser ablation method and its antibacterial properties" to Indonesian Journal of Chemistry. With the online journal management system that we are using, you will be able to track its progress through the editorial process by logging in to the journal web site:

Manuscript URL: https://jurnal.ugm.ac.id/ijc/author/submission/60344
Username: khumaeni

If you have any questions, do not hesitate to contact me. Thank you for considering this journal for publishing your valuable work.

Best regards,
Prof. Dr.rer.nat. Nuryono, MS
Indonesian Journal of Chemistry

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3. Editor and reviewers' comments (22 November 2020)

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Ali Khumaeni <khumaeni@fisika.fsm.undip.ac.id>

[IJC] Editor Decision

Dr. Adhi Dwi Hatmanto <adhi.dwi.h@mail.ugm.ac.id> Sun, Nov 22, 2020 at 8:10 AM
To: Dr Ali Khumaeni <khumaeni@fisika.fsm.undip.ac.id>

Dear Dr Ali Khumaeni,

We have reached a decision regarding your submission to Indonesian Journal of Chemistry, "Synthesis of colloidal silver Nanoparticles in various liquid media using pulse laser ablation method and its antibacterial properties".

Our decision is: Revisions Required

Comments of the reviewers can be seen in the bottom part of this email.

The revised paper has to be completed with the responses for the reviewer's comments, point by point, in table form at the beginning of the page of the revised paper. It is also required to highlight the revised parts with a different color of letters.

The revised paper has to be resubmitted in the system within three weeks.

Thank you for your intending to contribute to the journal and for giving us to read your work.

Best regards,

Dr. Adhi Dwi Hatmanto
Universitas Gadjah Mada
Phone +6281259897530
adhi.dwi.h@mail.ugm.ac.id

Reviewer A:

Additional Comment::
There is not that much new in the article. Also, the biological portion is not clearly discussed and is also not up to the mark.

Reviewer B:

Additional Comment::
(1) Title: The title should be edited, no point in writing nanoparticle with capital N
(2) Abstract: Not complete, important findings and conclusions (and perhaps the outlook) should be included.
(3) Introduction: The choice of using pulse laser ablation (PLA) in the preparation of AgNPs seems not justified well. More references are needed.
(4) Procedures: Did you synthesize tin oxide nanoparticles? Check. Antibacterial testing should be more detailed, readers may do not have a chance to read the original reference. Aquadest is not an English word. How were the dead bacteria measured/counted? Are there any related data?
(5) Results and discussion: The reaction mechanism of AgNPs should be included. Tables 1 and 2 could be combined. Details on the LSPR data concerning the particle size and shape were not there.
(6) Special note: Typos and grammatical errors are everywhere. S-V agreements errors are a lot. Be consistent, are using American or British English?

Reviewer C:

Additional Comment::
1) English written is good with minimal typo and grammatical errors.
2) It is better to include enough key information. e.g., summary results (major findings). However, there are no results for antibacterial properties, according to the title.
3) The introduction shows that the research work is engaging, concise and well structured.
4) One sentence in page 3 needs to be rephrased into passive terms.
5) Table 1 should be written after its text.
6) There is no text refer to Table 2.

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4. Balasan komentar Editor dan Reviewers

Title: Synthesis of colloidal silver Nanoparticles in various liquid media using pulse laser ablation method and its antibacterial properties

Dear Editor in Chief
Indonesian Journal of Chemistry

Thank you very much for reviewing our paper entitled "Synthesis of colloidal silver Nanoparticles in various liquid media using pulse laser ablation method and its antibacterial properties", which we have submitted to Indonesian Journal of Chemistry.

We have completely read your letter and feel happy that our manuscript is suitable for publication in Indonesian Journal of Chemistry after appropriate revisions.

In this letter, we would like to respond the comments from reviewers as below. Considering the comments from reviewers, we have made a final revision in our manuscript. The revision parts are shown in the revised manuscript using red letter.

We would like to thank you very much for your kindness.

Best regards
Ali Khumaeni et al.

Reviewer A:

Reviewer point #1: There is not that much new in the article. Also, the biological portion is not clearly discussed and is also not up to the mark.

Author response #1: We have added a novelty of our manuscript as written in the revised manuscript part Introduction. The part of revision is below,

It should be mentioned that the above reports are mainly used high-energy of 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 for the application of the produced silver nanoparticles for antibacterial agent. In this present work, we conducted a study on synthesis of AgNPs in various medium of polyvinylpyrrolidone (PVP), polyethylene glycol (PEG), and chitosan using the pulse laser ablation synthesis method. As mentioned above that the use of pulse laser ablation technique for preparation of AgNPs in this study is because this technique can produce high-purity nanoparticles without additional hazard chemical agents for medical application and human-related products.

For biological portion, we have added in the revised manuscript, part of Results and Discussion. The part of revision is below,

As reported here, the interaction mechanism between AgNPs and bacteria is as follow: the AgNPs attach on the surface of cell wall and membrane. After that, the AgNPs penetrates inside the cell and inducing cellular toxicity and oxidative stress by generation reactive oxygen species and free radicals, damaging intracellular structures and biomolecules.

Reviewer B:

Additional Comment::
Reviewer point #1: Title: The title should be edited, no point in writing nanoparticle with capital N

Author response #1: We have rewritten the title as in the revised manuscript. The part of revision is as follows: Synthesis of colloidal silver nanoparticles in various liquid media using pulse laser ablation method and its antibacterial properties

Reviewer point #2: Abstract: Not complete, important findings and conclusions (and perhaps the outlook) should be included.

Author response #2: We have revised the abstract as shown in the revised manuscript, part Abstract. The part of revision is as follows: Experimentally, a pulse Nd:YAG laser beam (1064 nm, 7 ns, 30 mJ) was directed using silver mirror and focused using quartz lens with a focal length of 30 mm on a silver metal plate placed in a petri dish containing liquid mediums for 120 minutes to produce colloidal silver nanoparticles. The results certified that All AgNPs have spherical shape with polidisperse size in all media including PVP, PEG, and chitosan. The smallest AgNPs have been produced in PVP medium with averaged smallest size of 11.62 nm. Furthermore, AgO compound also produced during synthesis process. Based on this result, PVP is preferable medium to produce AgNPs with smallest size and good stability. The produced silver nanoparticles have been successfully employed as antibacterial agent, which are experimentally demonstrated by using Escherichia coli and Staphylococcus aureus.

Reviewer point #3: Introduction: The choice of using pulse laser ablation (PLA) in the preparation of AgNPs seems not justified well. More references are needed.

Author response #3: We have added explanation about the choice of using pulse laser ablation in the revised manuscript, part of introduction. We have also added many references related to synthesis of silver nanoparticles using pulse laser ablation technique.

The part of revision is as follows: Many reports have been published on synthesis of AgNPs using PLA technique [10-15]. Tsuji et al. used pulse laser ablation technique to produce AgNPs in water medium under various wavelength of pulse laser including 1064 nm, 532 nm, and 355 nm [15]. However, AgNPs cannot disperse well and is not quit 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 ethanol medium to avoid agglomeration and precipitation [9].

Darroudi et al. employed gelatin solution to produce AgNPs having average diameter ranging from 9 nm to 15 nm. Al-Azawi et al. studied the effect of various liquid medium including deionized water, ethanol, and polyvinilpirrolidone (PVP) to 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 of 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 for the application of the produced silver nanoparticles for antibacterial agent. In this present work, we conducted a study on synthesis of AgNPs in various medium of polyvinylpyrrolidone (PVP), polyethylene glycol (PEG), and chitosan using the pulse laser ablation synthesis method. As mentioned above that the use of pulse laser ablation technique for preparation of AgNPs in this study is because this technique can produce high-purity nanoparticles without additional hazard chemical agents for medical application and human-related products.

Reviewer point #4: Procedures: Did you synthesize tin oxide nanoparticles? Check.

Author response #4: We have revised the sentence to be synthesis of silver nanoparticles as shown in the revised manuscript, part of procedure.

Reviewer point #5: Antibacterial testing should be more detailed, readers may do not have a chance to read the original reference.

Author response #5: We have added detail explanation about antibacterial testing as in the revised manuscript, part of procedure. The part of revision is as follows: The antibacterial test was made using AOAC 960.09 year 2013 with slightly modification [17]. The bacteria used were Escherichia coli (ATCC 8739) and Staphylococcus aureus (ATCC 6538). The temperature of incubation was 35^{o}C and the temperature of examination was 24^{o}C. A sample of 10 ppm AgNPs is inoculated with microorganims tests including E coli and S aureus with a concentration of 1.3 x 10^{6} UFC/ml and 1.1 x 10^{6} UFC/ml, respectively, at a contact time of 10 minutes. 1 ml of AgNPs were poured with 1 ml of test suspension. This mixture was then straited 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^{o}C. After incubation, the growth of the inoculated microorganism in each quadrant counted.

Reviewer point #6: Results and discussion: The reaction mechanism of AgNPs should be included. Tables 1 and 2 could be combined. Details on the LSPR data concerning the particle size and shape were not there.

Author response #6: We have added the information about reaction mechanism of AgNPs with bacteria as shown in the revised manuscript, part of Results and discussion. The interaction mechanism between AgNPs and bacteria is as follow: the AgNPs attach on the surface of cell *wall and membrane. After that, the AgNPs penetrates inside the cell and inducing cellular toxicity and oxidative stress by generation reactive oxygen species and free radicals, damaging intracellular structures and biomolecules.*

We have also combined Tables 1 and Table 2 as shown in the revised manuscript, part of Result and discussion.

Detail LSPR data concerning the particle size and shape have been added in the revised manuscript, part of Results and discussion, Application of AGNPs as an antibacterial agent.

Reviewer point #7: Typos and grammatical errors are everywhere. S-V agreements errors are a lot. Be consistent, are using American or British English?

Author response #7: We have revised typos and grammatical errors as in the revised manuscript.

Reviewer C:

Reviewer point #1: English written is good with minimal typo and grammatical errors.

Author response #1: Thank you very much for your appreciation.

Reviewer point #2: It is better to include enough key information. e.g., summary results (major findings). However, there are no results for antibacterial properties, according to the title.

Author response #2: We have revised the summary results or major findings including results for antibacterial properties in abstract as shown in the revised manuscript. Part of the revised abstract is follows: Experimentally, a pulse Nd:YAG laser beam (1064 nm, 7 ns, 30 mJ) was directed using silver mirror and focused using quartz lens with a focal length of 30 mm on a silver metal plate placed in a petri dish containing liquid mediums for 120 minutes to produce colloidal silver nanoparticles. The results certified that All AgNPs have spherical shape with polidisperse size in all media including PVP, PEG, and chitosan. The smallest AgNPs have been produced in PVP medium with averaged smallest size of 11.62 nm. Furthermore, AgO compound also produced during synthesis process. Based on this result, PVP is preferable medium to produce AgNPs with smallest size and good stability. The produced silver nanoparticles have been successfully employed as antibacterial agent, which are experimentally demonstrated by using Escherichia coli and Staphylococcus aureus.

Reviewer point #3: The introduction shows that the research work is engaging, concise and well structured.

Author response #3: Thank you very much for your comments.

Reviewer point #4: One sentence in page 3 needs to be rephrased into passive terms.

Author response #4: We have revised the sentence as in the revised manuscript following your comments.

Reviewer point #5: Table 1 should be written after its text.

Author response #5: We have revised the format as in the revised manuscript. We have wrote Table 1 after its text

Reviewer point #6: There is no text refer to Table 2.

Author response #6: We have combined Tbale 2 and Table 1 following the comment from other reviewer.

5. **Paper setelah proses revisi mempertimbangkan masukan Editor dan Reviewers**

Synthesis of colloidal silver nanoparticles in various liquid media using pulse laser ablation method and its antibacterial properties

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Abstract: *The silver nanoparticles (AgNPs) have been applied as an antibacterial agent in consumer products, cosmetics, medical tool coatings, and food industries. The technique generally used for synthesis of AgNPs is chemical reduction. However, the techniques involves high energy requirements and hazardous chemicals, which need further purification and are complicated in synthesis process due to the fact the chemical contamination. In this present work, AgNPs were synthesized in various medium 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 silver mirror and focused using quartz lens with a focal length of 30 mm on a silver metal plate placed in a petri dish containing liquid mediums for 120 minutes to produce colloidal silver nanoparticles. The results certified that All AgNPs have spherical shape with polidisperse size in all media including PVP, PEG, and chitosan. The smallest AgNPs have been produced in PVP medium with averaged smallest size of 11.62 nm. Furthermore, AgO compound was also produced during synthesis process. Based on this result, PVP is preferable medium to produce AgNPs with smallest size and good stability. The produced silver nanoparticles have been successfully employed as antibacterial agent, which are experimentally demonstrated by using Escherichia coli and Staphylococcus aureus. The result certified that the produced silver nanoparticles can effectively kill the bacteria with the killing percentage of 99.6 to 100 %.*

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

▪ INTRODUCTION

Silver nanoparticles (AgNPs) has 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 broaden 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 deliver, agent of anticancer drugs. Thus, synthesis of AgNPs has recently become an interest 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 a quiet good stability. However, the techniques involves high energy requirements and hazardous chemicals, which need further purification and are complicated in synthesis process due to the fact the chemical contamination. Hence, the AgNPs cannot be employed readily for medical purpose 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 purpose and human-related products.

The other technique for synthesis of AgNPs is 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 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 synthesis process and only needs pure metal and liquid medium such as deionized water. Furthermore, the pulse laser ablation technique has much simple experimental setup than the case of chemical technique. Many reports have been published on synthesis of AgNPs using PLA technique [10-15]. Tsuji et al. used pulse laser ablation technique to produce AgNPs in water medium under various wavelength of pulse laser including 1064 nm, 532 nm, and 355 nm [15]. However, AgNPs cannot disperse well and is not quit 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 ethanol medium to avoid agglomeration and precipitation [9]. Darroudi et al. employed gelatin solution to produce AgNPs having average diameter ranging from 9 nm to 15 nm. Al-Azawi et al. studied the effect of various liquid medium including deionized water, ethanol, and polyvinilpirrolidone (PVP) to 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 of 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 for the application of the produced silver nanoparticles for antibacterial agent.

In this present work, we conducted a study on synthesis of AgNPs in various medium 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 that the use of pulse laser ablation technique for preparation of AgNPs in this study is because this technique can produce high-purity nanoparticles without additional hazard chemical agents for medical application 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. Effect of liquid medium to the averaged sizes of produced AgNPs were 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 can effectively kill the bacteria with the killing percentage of 99.6 to 100 %.

EXPERIMENTAL SECTION

Materials

Silver metal plate with purity of 99,9 % and a dimension of 5x 10 x 20 mm^3 was used as a material target to produce AgNPs. Liquid medium for making colloidal AgNPs used were PVP, PEG, and Chitosan.

Instrumentation

The experimental set up 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.

Procedure

Synthesis of silver nanoparticles

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

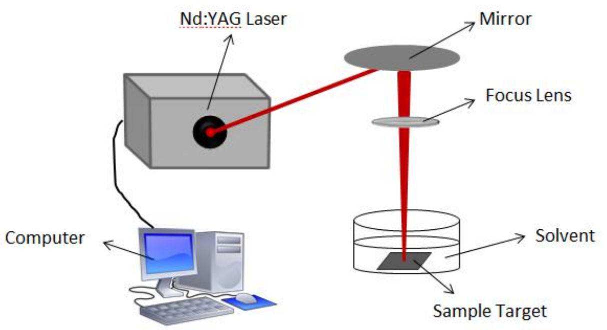

Fig. 1. Experimental setup

Characterization of silver nanoparticles

The produced colloidal AgNPs were the 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 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 year 2013 with slightly 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 examination was 24°C. A sample of 10 ppm AgNPs is inoculated with microorganims tests including E coli and S aureus with a concentration of 1.3 x 10^6 CFU/ml and 1.1 x 10^6 CFU/ml, respectively, at a contact time of 10 minutes. 1 ml of AgNPs were poured with 1 ml of test suspension. This mixture was then straited 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 counted.

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. Figure 2 shows photographs of colloidal AgNPs produced in (a) PEG, (b) PVP, and (c) chitosan using 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 high-purity Ag metal plate for 11 hours. It can be seen in the figure that the formation of colloidal AgNPs in the three liquid mediums is identified by 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 in elsewhere [18].

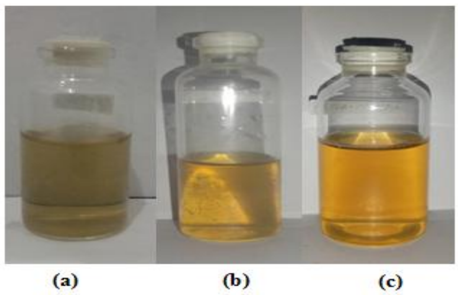

Fig 2. Colloidal AgNPs synthesized by pulse laser ablation technique in liquid medium (a) PEG, (b) PVP, and (c) Chitosan

The darker color of the colloid indicates a higher AgNPs [19]. The distinctive color of AgNPs is the result of the localized surface plasmon resonance (LSPR) phenomenon where silver material absorb. 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]. Figure 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. Figure 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.

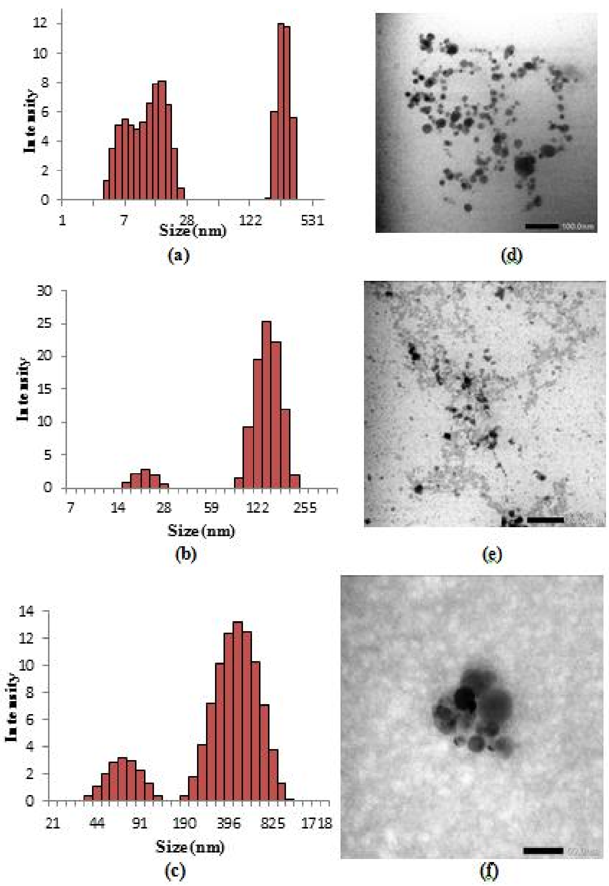

Fig. 3. Histograms of size distribution of AgNPs in (a) PVP, (b) PEG, (c) chitosan and AgNPs morphology in (d) PVP, (e) PEG, and (f) chitosan using PSA and TEM

From the size distribution data above, it is known that the colloidal AgNPs formed has two nanoparticle size distributions. This certified that the AgNPs colloids consists of polydisperse colloids that are 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 also 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, it 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 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 oscillates 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, the shape of the nanostructures, and the environment [28].

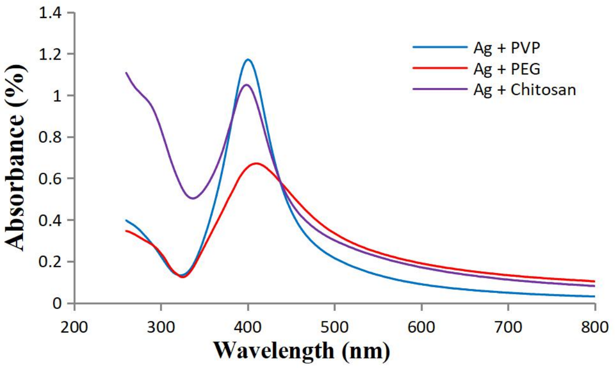

Fig. 4. UV-Vis spectrum of AgNPs colloid in PVP, PEG, and Chitosan liquid medium

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, the 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. In addition, 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 the 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.

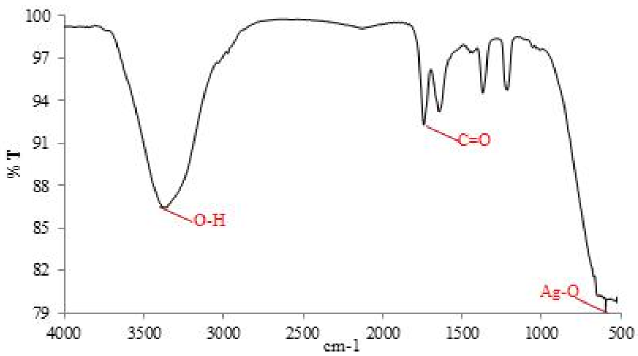

Fig. 5. IR transmittance spectrum of colloidal AgNPs on chitosan medium

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

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

Contamination time (s)	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
Staphyloccus aureus			
30	1.1×10^6	3.9×10^3	99.64
60	1.1×10^6	1.1×10^2	99.99

Table 1 shows the examination results of AgNPs as antibacterial agent in gram-negative Escherichia coli and gram-positive Staphyloccus aureus. For this experiment, number of initial bacteria is 1.3×10^6 CFU/ml and 1.1×10^6 CFU/ml, respectively. It can clearly be seen that after 30 s of AgNPs treatment to E coli bacteria, 100 % bacteria is dead and completely no bateria remain. For S aureus, 99.64 % S aeureus bacteria is dead. The dead bacteria increased up to 99.99 % when the interaction time of bacteria with AgNPs was much longer up to 60 s. This result certified that the produced colloidal AgNPs in PVP medium with a concentration of 10 mg/kg can effectively be used as an antibacterial agent. As reported here [31], the interaction mechanism between AgNPs and bacteria is as follow: the AgNPs attach on the surface of cell wall and membrane. After that, the AgNPs penetrates inside the cell and inducing cellular toxicity and oxidative stress by generation 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 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 pulse repetition rate of 10 Hz was employed as an energy source. The results certified that All AgNPs have spherical shape with polidisperse size in all media including PVP, PEG, and chitosan. The smallest AgNPs have been produced in PVP medium with averaged smallest size of 11.62 nm. Furthermore, AgO compound also produced during synthesis process. Based on this result, PVP is preferable medium to produce AgNPs with smallest size and good stability. In this present work, AgNPs have been succesfully applied as antibacterial agents in E coli and S aureus bacteria.

ACKNOWLEDGMENTS

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REFERENCES

[1] Kruszewski, M.,et al., 2011,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., Prina-Mello, A., 2018, R. Soc. Open Sci., 5(2)

[3] Chen, J. C., Lin, Z. H., Ma, X. X., 2003, Lett. Appl. Microbiol., 37(2), 105–108

[4] Natsuki, J., Natsuki, T., Hashimoto, Y., 2015, Int. J. Mater. Sci. Appl., 4(5), 325–332

[5] Bosetti, M., Massè, A., Tobin, E., Cannas, M.,(2002),Biomaterials, 23(3), 887–892
[6] Yeo, S.Y., Lee, H.J., Jeong, S.H., 2003, J. Mater. Sci., 38(10), 2143–2147
[7] Lkhagvajav, N., Ya, I Ş A., Çel, E İ K., Koizhaiganova, M., Sari, Ö., 2011,Journal of Nanomaterials and Biostructures, 6(1), 149–154
[8] Mishra, Y.K,et al., 2007, Scripta Materialia, 56, 629–632
[9] Valverde-alva, M A, et al., Applied Surface Science,355, 341–349
[10] Bae, C.H., Nam, S.H., and Park, S.M., 2002,Appl. Surf. Sci., 197–198, 628–63
[11] Boutinguiza, M, et al., 2015, Applied Surface Science, 336, 108–111
[12] Darroudi, M, et al., 2011, Journal of Alloys and Compounds, 509(4), 1301–1304.
[13] Mafune, F, et al., 2000, J. Phys. Chem. B, 104(39), 9111–9117
[14] Pyatenko, A, et al., 2004, Applied Physics A: Materials Science and Processing, 79(4–6), 803–806.
[15] Tsuji, T., 2002, Applied Surface Science, 202, 80
[16] Al-azawi, M. A, et al., 2016, Jurnal Teknologi, 3, 7–11
[17] Song, X., Vossebein, L., & Zille, A, 2019, Antimicrobial Resistance and Infection Control, 8(1), 1–14.
[18] Solomon, Sally D., et al., (2007), J. Chem. Educ.,84(2), 322–325
[19] Ahmed, B.S,et al., (2016), Cancer Oncol. Res., 4(3) 42–51
[20] Abadeer, Nardine S., Murphy, Catherine J., 2016, . Phys. Chem. C, 120(9),4691
[21] Evanoff Jr D.D and Chumanov G., Chem.Phys.Chem, pp. 1221–1231, 2005,
[22] Rodríguez-León,E. et al., Nanoscale Res. Lett., vol. 8, no. 1, pp. 1–9, 2013
[23] Nuraeni,W., Daruwati, I., E. M. W, and Sriyani,M. E., 2013, PTNBR- BATAN Bandung, 266–271
[24] Lichtenegger,T. and Pirker,S., 2018, Powder Technol., 325, 698–711
[25] Kim,S., Osone,M., Kim,T., Higashi,H., and Seto,T., 2017, KONA Powder Part. J., no. 34, 80–90
[26] Tejamaya,M., Merrifield, R. C., and Lead,J. R., 2012, Environmental Science and Technology.
[27] Tiyaboonchai,W., 2003, Naresuan Univ. J., 11(3), 51–66
[28] Minh Hiep,H., et al., Sci.,2007, Technol. Adv. Mater., 8(4), 331–338
[29] Chhatre,A., Solasa,P., Sakle,S., Thaokar,R., and Mehra,A., 2012, Colloids Surfaces A Physicochem. Eng. Asp., 404, 83–92,
[30] Hosseinpour-Mashkani,S. M. and Ramezani M., 2014, Mater. Lett., vol. 130, pp. 259–262
[31] Dakal, T. C., Kumar, A., Majumdar, R. S., Yadav, V., 2016, Front. Microbiol., 7, pp. 1831

6. **Bukti email mengirim komentar dan revisi manuskrip ke Jurnal (9 Desember 2020**

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[IJC] Editor Decision

Ali Khumaeni <khumaeni@fisika.fsm.undip.ac.id> Wed, Dec 9, 2020 at 10:25 PM
To: "Dr. Adhi Dwi Hatmanto" <adhi.dwi.h@mail.ugm.ac.id>, Khumaeni@gmail.com

Dear Dr Adhi Dwi Hatmanto

We would like to thank you very much for your kindness to send the comments from reviewers. We are very pleased to know that most reviewers give positive comments. We have revised the submitted manuscript following the reviewer comments.

We would like to send the response to reviewer comments and revised manuscript following reviewer suggestions as in the attached file. We have also uploaded the revised manuscript in the system.

We would like to thank you very much for your kindness.

Best regards
Ali Khumaeni

[Quoted text hidden]

2 attachments

Comments and responds to Reviewers FINAL 20201209.docx
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7. **Acceptance letter dari jurnal dari seluruh proses review (1 Januari 2021)**

webmail FISIKA universitas diponegoro

Ali Khumaeni <khumaeni@fisika.fsm.undip.ac.id>

[IJC] Editor Decision

Dr. Adhi Dwi Hatmanto <adhi.dwi.h@mail.ugm.ac.id> Fri, Jan 1, 2021 at 8:24 PM
To: Dr Ali Khumaeni <khumaeni@fisika.fsm.undip.ac.id>

Dear Dr Ali Khumaeni:

We have reached a decision regarding your submission to Indonesian Journal of Chemistry, "Synthesis of colloidal silver Nanoparticles in various liquid media using pulse laser ablation method and its antibacterial properties".

Our decision is to: Accept Submission

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Thank you for your valuable contribution to the journal.

Best regards,

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Reviewer A:

Additional Comment::
All the comments previously raised by the reviewers have been addressed. This paper could be accepted pending rigorous English improvement.

Reviewer B:

Additional Comment::
There are still a few typos that need to be corrected. Please recheck Table 1. Explanation(text) for Table 1 should be written first, followed by the Table 1 itself.

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8. Galley proof confirmation (16 Maret 2021)

Ali Khumaeni <khumaeni@fisika.fsm.undip.ac.id>

[IJC] Proofreading Acknowledgement (Author)

1 message

Dr. Adhi Dwi Hatmanto <adhi.dwi.h@mail.ugm.ac.id> Tue, Mar 16, 2021 at 7:12 PM
To: Dr Ali Khumaeni <khumaeni@fisika.fsm.undip.ac.id>

Dear Dr Ali Khumaeni,

Thank you for proofreading the galleys for your manuscript, "Synthesis of colloidal silver Nanoparticles in various liquid media using pulse laser ablation method and its antibacterial properties," in Indonesian Journal of Chemistry. We are looking forward to publishing your work shortly.

If you subscribe to our notification service, you will receive an email of the Table of Contents as soon as it is published. If you have any questions, please contact me.

Best regards,
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Synthesis of Colloidal Silver Nanoparticles in Various Liquid Media Using Pulse Laser Ablation Method and Its Antibacterial Properties

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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

Full Text:

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.

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King Saud Univ. Sci., 2[illegible] (4), 273–277.

[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.

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