Bukti Korespondensi
Journal of Nano Research

1. Draft manuskrip sebelum proses submission
2. Submission acknowledgment dari Journal of Nano Research (1 November 2020)
3. Editor and reviewers' comments (21 Desember 2020)
4. Balasan komentar Editor dan Reviewers
5. Paper setelah proses revisi mempertimbangkan masukan Editor dan Reviewers
6. Submission kedua setelah proses review (30 Desember 2020)
7. Acceptance letter dari jurnal dari seluruh proses review (3 Maret 2021)
8. Notifikasi publikasi dari Journal of Nano Researrch (30 Juli 2021)

1. Draft manuskrip sebelum proses submission

Synthesis of tin oxide nanoparticles by pulse laser ablation method using low-energy Nd: YAG laser as antibacterial agent

ANA QONA'AH[1], IIS NURHASANAH[1] and ALI KHUMAENI[1,a*]

[1]Departement of Physics, Faculty of Science and Mathematic, Diponegoro University, Semarang, Indonesia 50275

[a*]email, *khumaeni@fisika.fsm.undip.ac.id

Keywords: Pulse laser ablation; pulse laser ablation in liquid; tin oxide nanoparticles; Escherichia coli bacteria; disc diffusion method; antibacterial agents.

Abstract. The synthesis of tin oxide nanoparticles (SnNPs) was successfully carried out using the neodymium yttrium aluminum garnet (Nd: YAG) low power pulse laser ablation method. Synthesis was carried out using a laser frequency of 10 Hz with a variation of the liquid medium of distilled water and ethylene glycol which produced a brownish colloidal color. Characterization of tin oxide nanoparticles includes UV-Vis, EDX, FTIR, and TEM. UV-Vis characterization produced absorbance values in aquadest and ethylene glycol mediums of 1.314 a.u. and 1.119 a.u., respectively. TEM images show that the shape of tin oxide nanoparticles produced are spherical. Measurement of nanoparticle size distribution using image-J software obtained the average diameter size in the ethylene glycol medium 12.55 nm smaller than the size in the aquades medium which is 19.98 nm. The EDX spectrum analysis results show that there are only Sn and O atoms in colloidal tin oxide nanoparticles (SnNPs). FTIR results show the formation of SnO_2 (SnNPs) spectrum at wave number 629.03 cm^{-1}. Testing the antibacterial activity of Escherichia coli on SnNPs using a disc diffusion method with nanoparticle concentrations of 10 ppm, 20 ppm and 30 ppm. The test results of the disc diffusion shows the diameter of inhibition zone (DIZ) in sequence 6.50 mm, 6.75 mm, and 9.50 mm. The higher the concentration of SnNPs given, the greater the ability to degrade and inhibit bacteria.

Introduction

Tin (Sn; Z = 50) is a metal element that has many benefits in life. Along with the development of science, metal elements, especially tin, began to be synthesized into nanoparticles (NPs) to optimize their utilization. Nanoparticles are small materials that have sizes ranging from 1 to 100 nm and can be classified based on their nature, shape, and size [1]. SnNPs are used as photocatalysts, reducing aromatic compounds, gas sensing properties, sensors, plant growth like spinach. Whereas in the health sector, SnNPs are used as antibacterial, antioxidants, anti-cancer and weight gain [2].

Bacteria are a contributor to disease. The environment contaminated by bacteria can affect human health such as the Escherichia coli bacteria. E. coli bacteria easily attach to food, especially to undeveloped foods. If the food consumed is continuously contaminated with E. coli bacteria, it will have an impact on diseases such as diarrhea. Diarrhea is a disease that causes a person to defecate with a soft texture, even in the form of water in a small period of time, but it occurs more than 3 times [3]. Therefore there is a need for technology and ingredients as antibacterial agents to reduce the risk of the disease. Along with the development of science, antibacterial agent research is increasingly sophisticated and many discoveries of materials that can be used as antibacterial agents, one of which is tin metal (Sn).

Vidhu and Philip studied the antibacterial activity of SnNPs against E. coli bacteria. It was observed that bactericidal activity was inhibited as nanoparticle concentrations increased and it was therefore stated that higher SnNPs concentrations had more effect on bactericides, meaning that the higher the concentration of SnNPs, the greater the ability of these substances to kill or inhibit bacterial growth [4].

Several conventional methods have been used for the synthesis of SnNPs, including the hydrothermal method, the precipitation method, the sol gel method, hydrolysis, and chemical reduction [5]. But, the methods these require complicated procedures and require chemicals as surfactants. This causes the NPs produced to have low purity so that it is not appropriate to be applied in the health sector.

Another method used for the synthesis of NPs is pulse laser ablation (PLA). In this method high power PLA is focused on the metal surface placed in the liquid to produce colloidal NPs. Compared to chemical methods, the PLA method does not require complicated processes, is environmentally friendly, and can produce high purity NPs because it does not require surfactants in the synthesis process [6]. The laser ablation process pulse on the surface of the tin plate will induce the formation of SnNPs colloids without the use of organic surfactants, resulting in high purity NPs [7,8]. However, this research has not yet tested the characteristics of the effect of liquid media on the NPs produced, so this research will do the synthesis of SnNPs using PLA method in aquades and ethylene glycol media.

The research was carried out testing of SnNPs colloids as antibacterial agents of E. coli. SnNPs is one of the right ingredients as an antibacterial agent using the PLA method, because tin has the characteristics of a softer material so that it is easily synthesized by the PLA method. SnNPs colloids will quickly form so that they can produce colloids NPs with higher concentrations in a more efficient time.

Experimental

Materials. The material used for this study consisted of tin plates are used as target material in the manufacture of colloidal SnNPs with a purity of 99.95%, aquadest and ethylene glycol 5 M as liquid media for making colloidal SnNPs.

Instrumentation. The tools used for this study consisted of neodymium yttrium aluminum garnet laser (Nd: YAG) (New Polaris II model, base wavelength 1064 nm, maximum repetition rate of 20 Hz) as the energy source used and laser Exec II software, to set laser parameters (energy, repetition rate).

Synthesis of tin oxide nanoparticles. The natural rubber is ground with an open mill until it becomes soft (15 min). Synthesis of colloidal tin oxide (SnO_2) nanoparticles using an Nd: YAG pulse laser with a wavelength of 1064 nm and a pulse width of 7 ns. The synthesis of colloidal SnNPs was varied in liquid media using two types of liquids, namely distilled water and 5 *M* ethylene glycol solution.

The plates used for the synthesis were pure tin plates (99.95%) each placed in a petri dish containing a liquid medium in the form of distilled water and 5 M ethylene glycol liquid medium.The laser is operated in pulse mode, the laser beam is deflected by a silver mirror towards convex lens with a focal length of 3 cm, which functions to focus the laser beam onto the target surface at the base of the petri dish. 60 minutes of laser fire with a laser energy of 40 mJ, 10 Hz laser beam frequency to produce colloid samples of SnNPs.

Characterization of tin oxide nanoparticles. Several tests were carried out to characterize the colloid nanoparticles obtained. To determine the spectrum and absorbance level of tin oxide nanoparticles colloid (SnO_2), a UV-Visible Light Spectroscopy (UV-Vis) test was performed. The UV-Vis test was carried out by inserting 3-4 ml of colloidal tin oxide nanoparticles (SnO_2) into the cuvette, the cuvette containing colloidal tin oxide (SnO_2) nanoparticles was placed into the instrument and the data could be read after the UV-Vis instrument was operated. The Energy Dispersive X-Ray (EDX) test was carried out to determine the compounds contained in colloidal tin oxide nanoparticles (SnO_2) by dropping colloidal tin oxide (SnO_2) nanoparticles on a silicon carbide (SiC) plate measuring 0.5 cm x 0.5 cm and wait for the silicon carbide (SiC) plate to dry. The Fourier Transform Infra Red (FTIR) Spectrophotometer test was also carried out with the aim of identifying functional groups and compounds contained in tin oxide (SnO_2) nanoparticles colloid.

To see the morphology and size of tin oxide (SnO_2) nanoparticles, a Transmission Electron Microscope (TEM) test was performed. The image results from the TEM test were then processed using imageJ software to determine the size distribution of the resulting tin oxide (SnO_2) nanoparticles.

Antibacterial testing. Testing of SnNPs as an antibacterial agent was carried out using the disc diffusion method. The samples to be tested were based on variations in concentration using distilled water media with a concentration of 10 ppm, 20 ppm, and 30 ppm respectively. The disc diffusion method is carried out by measuring the diameter of the inhibition zone (DIZ), which is an indication of an inhibitory response to bacterial growth by an antibacterial compound in the extract. Before making the test medium, an important first step is to determine the number of bacteria in the media (disc). The bacterial suspension was diluted to have a turbidity level or optical density of 0.5 Mc Farland. The standard of 0.5 Mc Farland is the number of bacteria in 1 milliliter of suspension estimated at 1.5 x 10^8 bacteria. The test medium was made by mixing the agar nutrients with a diluted suspension of Escherichia coli bacteria, the media was stirred until evenly distributed then allowed to dry and solidify (plate medium). Furthermore, the filter paper that has been sterilized is then immersed in each colloidal SnNPs with various concentrations of 10 ppm, 20 ppm and 30 ppm. The filter paper is then placed on the test media. The test media was put into an incubator at 37°C for 18-24 hours and the inhibition zone diameter was observed [9].

Results and Discussion

Effect of liquid medium on tin oxide nanoparticles (SnNPs). Synthesis results in aquadest and 5 M EG media produced brownish colored SnNPs seen in Fig. 1 and 2 (a), according to the results of the research of Torres-Mendieta et al [10]. After settling for one day, the colloidal color of SnNPs begins to fade to light brown. On the other hand also found very small particles at the bottom of the bottle as shown in Fig. 1 and 2 (b). Two days after settling after being synthesized, the colloidal color fades to become almost clear and it appears that more and more particles are at the bottom of the bottle (Fig. 1 and 2 (c)). Fig. 1 and 2 (d) are colloids which are left to stand six days after being synthesized, the color of the colloids becomes very clear and more and more particles are found at the bottom of the bottle. Particles produced from the bottom of the bottle are the result of agglomeration and precipitation of nanoparticles dispersed in a liquid medium.

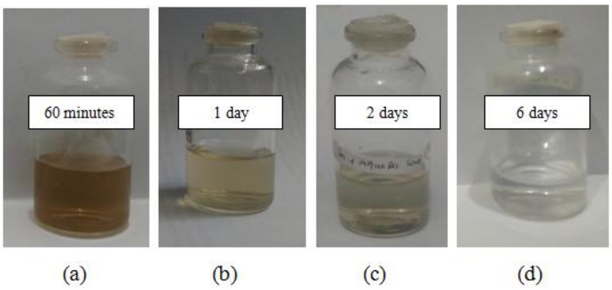


Fig. 1. Colloidal SnNPs in aquadest medium.

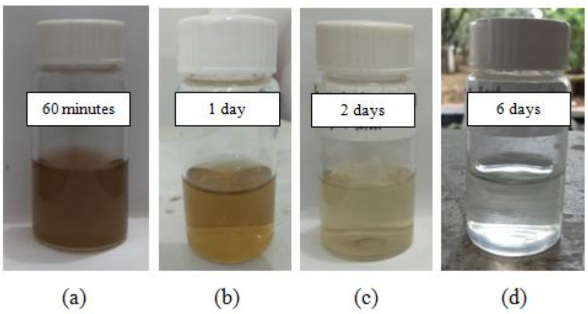


Fig. 2. Colloidal SnNPs in 5 M ethylen glycol medium.

Shortly after the laser hits the target, atoms and ions are affixed from the surface of the target material to move randomly at high speed and produce high-temperature plasma. Atoms and ions move randomly so quickly that they collide with one another, the movement of atoms and ions is called Brownian motion. The hotter the temperature in the target material area, the higher the frequency of collisions between atoms / molecules and ions. The frequency of collisions between atoms and molecules increases, so the more the number of nanoparticles stick together and agglomerated they will form larger NPs. Particles with a larger size that settles at the bottom of the bottle will make the concentration of NPs dispersed in the liquid media decreases (becomes clearer) [11].

Analysis of Colloidal Absorption Spectrum SnNPs using UV-Vis. UV-Vis analysis was carried out to determine the intensity of the produced SnNPs colloids. Fig. 3 shows the colloidal absorption spectrum of SnNPs in aquadest and 5 M EG media.

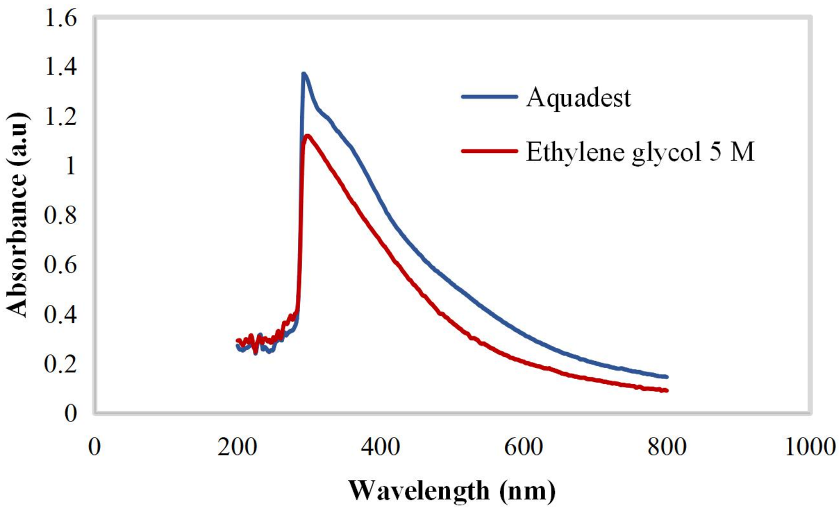
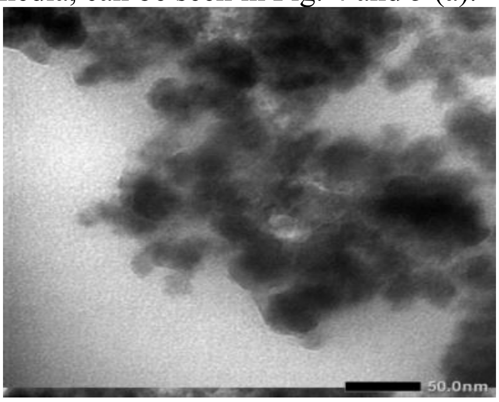


Fig. 3. Colloidal absorption spectrum of SnNPs.

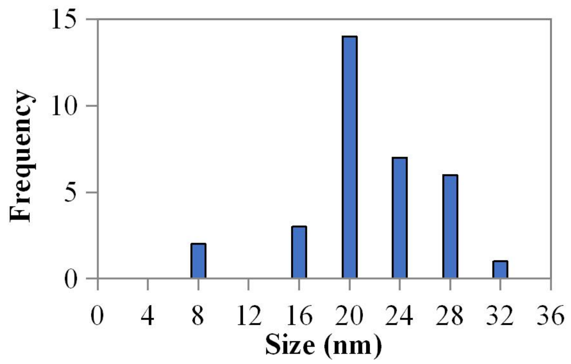
As shown in Fig. 3, both spectra have a single absorbance, where colloidal SnNPs in aquadest media have a maximum absorbance of 1.37 a.u. whereas in the 5 M EG media it has a maximum absorbance of 1.119 a.u.

With the same duration of ablation and repetition rate, the 5 M EG media has a lower absorption intensity than in aquades media. That is because the laser beam before hitting the surface of the lead target is blocked by the EG molecule and the laser energy that hits the target results in the target material being agglomerated being less, so that the density of particles formed in the solution is reduced [12].

Morphological analysis of SnNPs. TEM images showing SnNPs synthesized by the PLA method in spherical aquades and 5 M EG media, can be seen in Fig. 4 and 5 (a).

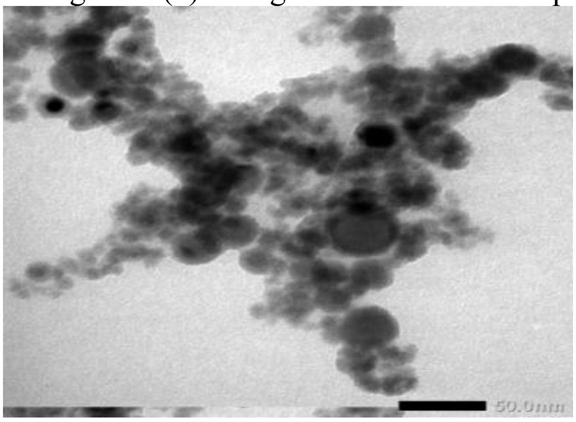


(a)

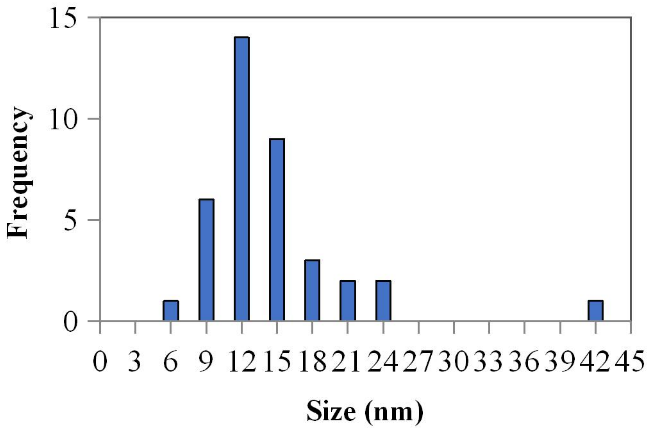


(b)

Fig. 4. (a) TEM image and (b) histogram SnNPs size in aquadest medium.



(a)



(b)

Fig. 5 (a) TEM image and (b) histogram SnNPs size in ethylene glycol medium.

From Fig. 4 (a) it can be seen that nanoparticles in aquades media are more clumped than in 5 M EG media, Figure 5 (a). This shows that SnNPs in distilled water media tend to be more aggregated and it is not easy to differentiate between one nanoparticle and another [13]. As a result of Brown's motion a particle moves quickly, so it has a tendency to clot. Therefore, the final nature of nanofluids does not depend on the primary particles but on the hydrodynamic size of the agglomeration of the formed nanoparticles [10].

Adding EG to distilled water (which is a 5 M EG solution) can reduce the average diameter of SnNPs from 19.98 nm to 12.55 nm. This decrease in the average diameter of the nanoparticles is due to the fact that in 5 M EG liquid media the surface of the SnNPs is coated or blocked by EG molecules which make the nanoparticles scattered so well that it can help reduce the attachment of particles to one another which can result in the size of the nanoparticles becoming larger [13].

Identification of SnNPs colloidal. Tin oxide nanoparticles (SnNPs) can be identified by their elements and compounds using Electron Dispersive X-ray (EDX) and Fourier Transform Infra Red (FTIR) devices. Preparation of EDX samples was carried out by colloidal SnNPs in distilled water media dripped on silicon carbide (SiC) plates measuring 0.5 × 0.5 cm, then dried at 30°C for 24 hours. Fig. 6 shows the EDX spectrum for colloidal SnNPs.

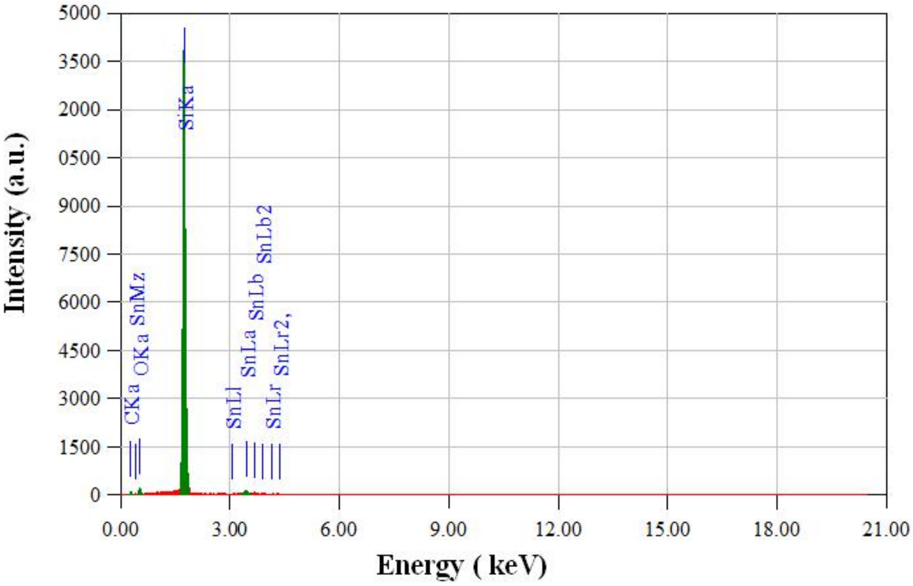


Fig. 6. SnNPs EDX spectrum.

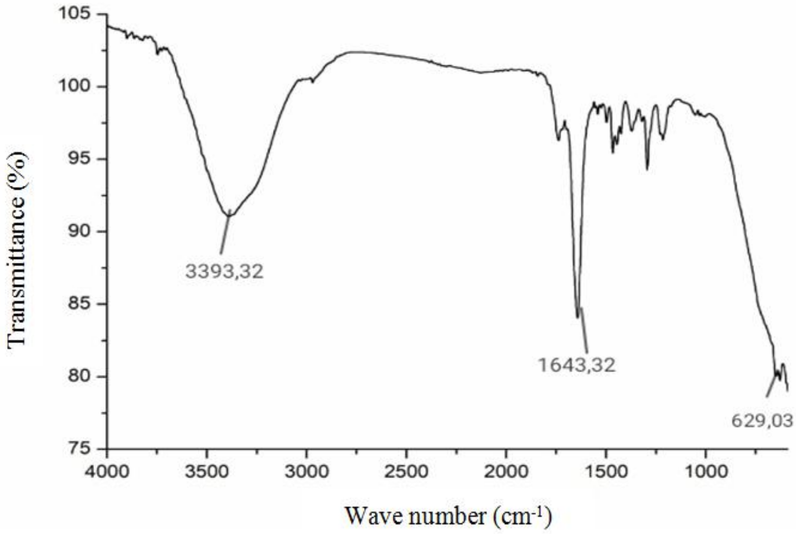


Fig. 7. SnNPs FTIR spectrum.

EDX analysis shows the presence of silicon (Si), tin (Sn), oxygen (O), and carbon (C) elements in the sample. Tin was detected in the spectrum because the material used for the manufacture of colloidal SnNPs was a high purity tin plate (99.95%). Meanwhile, the presence of O in the spectrum indicates the formation of SnO_2 compounds (SnNPs). Si and C peaks in the spectrum come from the substrate used for testing, the SiC plate. It also shows that colloidal SnNPs made in this study have high purity because no foreign elements are found in the EDX spectrum.

FTIR analysis is also used to identify compounds of SnO_2 (SnNPs). Fig. 7. shows the FTIR spectrum of colloidal SnNPs synthesis. The absorption peaks at 3393.32 cm^{-1} and 1643.32 cm^{-1} were detected from aquadest liquid medium hydroxyl groups. Bands that appear in the range 400-700 cm^{-1} are Sn-O antisymmetric characteristics. The presence of the 629.03 cm^{-1} peak in the synthesized sample confirms the presence of SnO_2 [14]. Abruzzi, Dedavid, and Pires also wrote to his journal that the peak of transmittance between 600-660 cm^{-1} is characteristic of SnO_2 [15].

Antibacterial activity of E. coli. Antibacterial activity test using the disk diffusion method was carried out by measuring the diameter of the inhibition zone (DIZ), which is an indication of the response to inhibiting bacterial growth by an antibacterial compound in the extract [9]. Positive control media using terramycin which is an antibiotic and contains strong antimicrobial effects. Terramycin is a versatile and effective antibiotic to treat infections caused by gram-positive and gram-negative bacteria such as E. coli. While the negative control media using Dimethyl Sufoxide (DMSO) solution which has no activity against the test bacteria.

Table 1. Test results for E. coli bacteria activity.

No	Sample	Inhibition zone diameter (mm)
1	Negative control	0
2	Positive control	38.00
3	10 ppm	6.50
4	20 ppm	6.75
5	30 ppm	9.50

As the results of the antibacterial activity test using the disk diffusion method in Table 1 shows that the negative control with DIZ 0 mm, shows that the negative control using DMSO is resistant, which means the bacteria cannot be killed. Positive control using terramycin on E. coli bacteria showed a pretty good antibacterial effect, DIZ was quite large at 38 mm, this indicates that there was an inhibition of bacterial and bacterial activity in the death phase.

Colloidal SnNPs against the bacteria tested, the largest DIZ occurred in colloidal SnNPs with a concentration of 30 ppm, the next 20 ppm, and the smallest DIZ at a concentration of 10 ppm. This shows that the higher the concentration of SnNPs, the inhibition of bacterial activity will also increase [4].

Conclusion

Based on the results obtained in this study, several conclusions can be formulated including the synthesis of SnNPs using the PLA Nd: YAG method successfully carried out in aquades and EG 5 M media with a repetition rate of 10 Hz resulting in brownish colloidal SnNPs. The results of EDX and FTIR characterization showed that the synthesized SnNPs colloids consisted of SnO_2 compounds. The morphology of SnNPs by TEM images in 5 M EG spherical media with an average diameter of 12.55 nm is smaller than the diameter size in aquades media which is 19.98 nm. The results of antibacterial activity tests using the disk diffusion method showed the largest DIZ sequentially at SnNPs concentrations of 30 ppm, 20 ppm, and 10 ppm were 9.5 mm, 6.75 mm and 6.50 mm.

Acknowledgment

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2. **Submission acknowledgment dari Journal of Nano Research (1 November 2020)**

webmail FISIKA universitas diponegoro

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

Paper «Synthesis of Tin Oxide Nanoparticles by Pulse Laser Ablation Method Using Low-Energy Nd: YAG Laser as Antibacterial Agent» has just been submitted by Ali Khumaeni

JNanoR <9780000010698@scientific.net> Sun, Nov 1, 2020 at 2:26 PM
Reply-To: authors@scientific.net
To: "Dr. Ali Khumaeni" <khumaeni@fisika.fsm.undip.ac.id>

Dear Ali Khumaeni

Paper titled «Synthesis of Tin Oxide Nanoparticles by Pulse Laser Ablation Method Using Low-Energy Nd: YAG Laser as Antibacterial Agent» has successfully been submitted in the book «JNanoR». Corresponding editors will keep you updated about the status of your paper.

Thank you for your contribution.

Best regards,
Team Scientific.Net

p.s. this is information email only. Please don't reply it unless misconduct or conflict of interest is an issue.

3. Editor and reviewers’ comments (21 Desember 2020)

webmail FISIKA universitas diponegoro

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

"JNanoR" Manuscript "Synthesis of Tin Oxide Nanoparticles by Pulse Laser Ablation Method Using Low-Energy Nd: YAG Laser as Antibacterial Agent"

1 message

JNanoR <9780000010698@scientific.net> Mon, Dec 21, 2020 at 4:36 PM
Reply-To: JNanoR <9780000010698@scientific.net>
To: "Dr. Ali Khumaeni" <khumaeni@fisika.fsm.undip.ac.id>

Dear Dr. Ali Khumaeni,

Based on the reports of the referees, the Editors have determined that your article «Synthesis of Tin Oxide Nanoparticles by Pulse Laser Ablation Method Using Low-Energy Nd: YAG Laser as Antibacterial Agent» requires revision before it can be published in the «JNanoR» . To access the referees' reports and submit your revised article, please enter the publisher's website https://www.scientific.net and log in using the credentials below:
Username : khumaeniali
Password : faiq2702

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Please check the Review(s), revise your manuscript and upload a new version. The new paper must be uploaded over the first version, just click on your paper title and upload the new version (mandatory).

The revised manuscript is uploaded over the earlier version, thus replacing it.

If you have any questions or wish to comment on your revisions, please e-mail the Editor.

Best regards,
Dr. Lina Kieush
Managing Editor Scientific.net

4. Balasan komentar Editor dan Reviewers

Title: Synthesis of tin oxide nanoparticles by pulse laser ablation method using low-energy Nd: YAG laser as antibacterial agent

Dear Editor in Chief
Journal of Nano Research

Thank you very much for reviewing our paper entitled "Synthesis of tin oxide nanoparticles by pulse laser ablation method using low-energy Nd: YAG laser as antibacterial agent", which we have submitted to Journal of Nano Research.

We have completely read your letter and feel happy that our manuscript is suitable for publication in Journal of Nano research 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 #1:

Reviewer point #1: Please check the grammatical and punctuation mistakes. The English structure must improve.

Author response #1: Thank you very much for the review, the grammar and punctuation errors have been fixed.

Reviewer point #2: In abstract; Why chose SnO_2 as source of antibacterial nanoparticles instead of other types of noble metal particles like nanoAg?

Author response #2: SnO_2 nanoparticles are one of the agents used as antibacterial instead of other metals such as Ag, Zn, and Cu. Compared to other metal such as Ag, Zn, and Cu, synthesis of SnO_2 nanoparticles from the Sn metal using pulse laser ablation method is much easier because the Sn metal is much softer compared to the Ag, Zn, and Cu metals, thus enable to produce colloidal nanoparticles with higher concentration at the same time. Furthermore, SnO_2 nanoparticles can effectively be used as antibacterial agent for both gram negative and gram positive bacteria.

Reviewer point #3: Rewrite the last sentence of abstract.

Author response #3: We have rewritten the last sentence in the abstract as follows : From the experimental results, it shows that the higher the concentration of SnNPs given, the greater the ability to degrade and inhibit bacteria. Therefore, SnNPs is one of the right ingredients as an antibacterial agent using the PLA method, because Sn has the characteristics of a softer material so that it is easily synthesized by the PLA method. SnNPs colloids will quickly form so that they can produce colloids NPs with higher concentrations in a more efficient time.

Reviewer point #4: Rewrite the 3rd paragraph of Introduction parts.

Author response #4: We have rewritten he 3rd paragraph of Introduction parts as follows: Nanotechnology is a known field of research since the last century. Various revolutionary developments in the field of nanotechnology have been carried out, one of which is in the medical field. Nanotechnology produces materials of various types at the nanoscale level referred to as nanoparticles. The importance of nanoparticles is realized that the particle size can affect the physiochemical properties of a substance, especially for the health sector [1].

Reviewer point #5: The last sentence of 4th paragraph needs citation with the appropriate reference (s).

Author response #5: We have added the citation to the last sentence in paragraph 4.

Reviewer point #6: Please merge 4th paragraph with 5th paragraph.

Author response #6: We've combined the 4th paragraph with the 5th paragraph.

Reviewer point #7: Please check the keywords based on the Tittle and Introduction parts.

Author response #7: We have checked the keywords by title and introduction, and we have rewritten the keywords.

Reviewer point #8: In materials part: all substances must explain clearly with the related information. please check some papers.

Author response #8: We have added an explanation based on the relevant information about all the substances present in the material section.

Reviewer point #9: In instrumentation parts: all instruments (like TEM, UV-Vis etc.) must explain clearly with the related information.

Author response #9: We have added an explanation based on the relevant information about all the instruments present in the instrumentation parts

Reviewer point #10: In Characterization of tin oxide nanoparticles part: please rewrite this paragraph based on the routines of paper structure. Please check some related papers.

Author response #10: We have rewritten based on a related paper in Characterization of tin oxide nanoparticles part.

Reviewer point #11: Could you please explain more this part? What is the differences between 1.119 a.u and 1.37 a.u?

"As shown in Fig. 3, both spectra have a single absorbance, where colloidal SnNPs in aquadest media have a maximum absorbance of 1.37 a.u. whereas in the 5 M EG media it has a maximum absorbance of 1.119 a.u".

Author response #11: The difference from the maximum absorption shows that in the aquadest medium 1.37 a.u. has a density greater than 5 M ethylene glycol 1.119 a.u., meaning that more nanoparticles are formed in the aquadest medium so that the maximum absorption is higher. Why the maximum uptake of aquadest is higher than in ethylene glycol 5 M, is explained in the result and discussion in the 3rd paragraph of the last sentence.

Reviewer point #12: In the morphological analysis of SnNPs: Morphology analysis is more related to SEM/FESEM instrument. However, change the images with more transparent one. Meanwhile, please calculate SD for every histogram.

Author response #12: We have changed the morphological image to a more transparent one, and have calculated the SD and we have added a table related to the SD calculation and the histogram mean diameter size in Table 1.

Reviewer point #13: Please write the abbreviated form of the words for the first time and then use it. (like page 5 the second paragraph for ethylene glycol).

Author response #13: We have changed the abbreviation for ethylene glycol to the original words and there is no abbreviation for ethylene glycol.

Reviewer point #14: Move this part to characterization part: “Preparation of EDX samples was carried out by colloidal SnNPs in distilled water media dripped on silicon carbide (SiC) plates measuring 0.5 × 0.5 cm, then dried at 30^{o}C for 24 hours.”

Author response #14: We've moved that section to the characterization section.

Reviewer point #15: Please add the table of the EDX information to the EDX part explanation.

Author response #15: We have added an information table regarding EDX data to Table 2.

Reviewer point #16: Rewrite this sentence again; “Abruzzi, Dedavid, and Pires also wrote to his journal that the peak of transmittance between 600-660 cm^{-1} is characteristic of SnO_2”.

Author response #16: We have rewritten the sentence by removing the author's name referring to the citation, in the 8th paragraph of Results and Discussion.

Reviewer point #17: Conclusions part: UV-Vis result also must be include.

Author response #17: We have added the UV-Vis results in the Conclusion section, in the 2nd sentence.

Reviewer #2:

Reviewer point #1: Figures 4 and 5: Write the units of Frequency (Unit (%)is not mentioned in the Y axis).
Author response #1: Thank you very much for the review, we have added the frequency unit (%) in Figures 4 and 5.

Reviewer point #2: Please note that the reference list and the whole manuscript must conform strictly to the Guide for Authors.
Author response #2: We've made sure the reference list matches the Guidelines for Authors.

Other comments: No further remarks.
Author response #3: Thank you very much for your positive review of our manuscript submitted to Journal of Nano Research

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

Synthesis of tin oxide nanoparticles by pulsed laser ablation method using low-energy Nd: YAG laser as an antibacterial agent

ANA QONA'AH, IIS NURHASANAH and ALI KHUMAENI[a*]

Department of Physics, Faculty of Science and Mathematics, Diponegoro University, Semarang, Indonesia 50275

[a*]email: khumaeni@fisika.fsm.undip.ac.id

Keywords: Tin oxide nanoparticles; antibacterial agent; pulse laser ablation method; Nd:YAG laser

Abstract. Tin oxide nanoparticles (SnNPs) are very useful to be employed as an antibacterial agent for both gram-positive and gram-negative bacteria. In this present work, the synthesis of SnNPs was successfully carried out using the neodymium yttrium aluminum garnet (Nd:YAG) laser with a wavelength of 1064 nm, pulse duration of 7 ns, and a laser frequency of 10 Hz. Experimentally, a pulse Nd:YAG laser was directed and focused on a high-purity tin (Sn) metal, immersed in various liquid media including pure water and ethylene glycol. A brownish colloidal colour was produced both in pure water and ethylene glycol liquid media. Characterizations of tin oxide nanoparticles were made using UV-Vis, EDX, FTIR, and TEM. UV-Vis characterization produced absorbance values in pure water and ethylene glycol media of 1.314 a.u. and 1.119 a.u., respectively. TEM images show that the shape of tin oxide nanoparticles produced is spherical. Measurement of nanoparticle size distribution was made using image-J software and the average diameter of nano-size in the ethylene glycol medium is 12.55 nm, which is smaller than the size in the pure water of 19.98 nm. The EDX spectrum analysis results show that there are only Sn and O atoms in colloidal tin oxide nanoparticles (SnNPs). FTIR results show the formation of tin oxide (SnO_2) spectrum at the wavenumber of 629.03 cm^{-1}. The produced colloidal SnNPs were then applied as an antibacterial agent of E. coli using the disk diffusion method. Results certified that various concentrations of SnNPs of 10 ppm, 20 ppm, and 30 ppm gain the diameter of inhibition zone (DIZ) in sequence 6.50 mm, 6.75 mm, and 9.50 mm. Based on these experimental results, it shows that the higher the concentration of SnNPs given, the greater the ability to degrade and inhibit bacteria.

Introduction

Tin (Sn; Z = 50) is a metal element that has many benefits in life. Tin metal is overwhelmingly available in Indonesia because Indonesia has huge tin metal resources, especially in Bangka Belitung Province. Along with the development of science, metal elements, especially tin, began to be synthesized into nanoparticles (NPs) to optimize their utilization. Nanoparticles are small materials that have sizes ranging from 1 to 100 nm and can be classified based on their nature, shape, and size [1]. SnNPs are used as photocatalysts, reducing aromatic compounds, gas sensing properties, sensors, plant growth like spinach. In the health sector, SnNPs are used as an antibacterial, antioxidants, anti-cancer, and weight gain [2].

Bacteria are a contributor to disease. The environment contaminated by bacteria such as the Escherichia coli bacteria can affect human health. E. coli bacteria easily attach to food, especially to undeveloped foods. If the food contaminated with E. coli bacteria is consumed continuously, it will have an impact on diseases such as diarrhea. Diarrhea is a disease that causes a person to defecate with a soft texture, even in the form of water in a small period, but it occurs more than 3 times [3]. Therefore there is a need for technology and ingredients as antibacterial agents to reduce the risk of the disease. Along with the development of science, antibacterial agent research is increasingly sophisticated and many discoveries of materials that can be used as antibacterial agents, one of which is tin metal (Sn).

Nanotechnology has become an interesting field of research since the last century. Various revolutionary developments in the field of nanotechnology have been carried out, one of which is in the medical field. Nanotechnology produces materials of various types at the nanoscale level referred to as nanoparticles. The importance of nanoparticles is realized that the particle size can affect the physicochemical properties of a substance, especially for the health sector [1].

Several conventional methods have been used for the manufacture of nanoparticles, especially tin oxide nanoparticles (SnNPs), including the hydrothermal method, precipitation method, sol-gel method, hydrolysis, and chemical reduction. But, the methods require complicated procedures and require chemicals as surfactants. This causes the NPs produced containing low purity so that it is not appropriate to be applied in the health sector [4]. Another method used for the synthesis of NPs is the pulsed laser ablation (PLA) method. In this method, high power pulse laser beam is focused on the metal surface placed in the liquid to produce colloidal NPs. Compared to chemical methods, the PLA method does not require complicated processes, is environmentally friendly, and can produce high purity NPs because it does not require surfactants in the synthesis process [5]. The bombardment of the pulse laser beam on the surface of the tin plate will induce the formation of SnNPs colloids without the use of organic surfactants, resulting in high purity SnNPs [6,7]. However, few studies on the synthesis of SnNPs have been made using the PLA method, especially the study on the characteristics of SnNPs in various liquid media. Therefore, in this present study, the synthesis of SnNPs was conducted using PLA method in various liquid media of pure water and ethylene glycol media. The produced colloidal SnNPs were characterized using UV-Vis, EDX, FTIR, and TEM to know the optical characteristics, atomic and compound composition, and morphological characteristics. Finally, the produced SnNPs were employed as an antibacterial agent of Escherichia coli using the disk diffusion method.

Experimental

Materials and Instrumentation. The materials used in this study were high-purity tin metal plate (99.95%, Nilaco, Japan), high-purity water, and Ethylene glycol (5 M, Wako 055-00996, Japan). The experimental tools used in this work consisted of neodymium yttrium aluminum garnet laser (Nd: YAG) (New Polaris II model, base wavelength 1064 nm, the pulse width of 7 ns, the maximum repetition rate of 10 Hz) as the energy source used and laser Exec II software, to set laser parameters (energy, repetition rate). For material characterizations, various instruments were used including The UV-Visible Light Spectroscopy (UV-Vis, Merck's Pharo 300 Spectroquant Spectrophotometer), the Energy Dispersive X-Ray (EDX, JEOL JSM-6510LA SEM-EDX), the Fourier Transform Infra-Red (FTIR) Spectrophotometer (FTIR Perkin Elmer Spectrum Version 10.4.00), and the Transmission Electron Microscope (TEM, JEOL JEM 1400).

Synthesis of tin oxide nanoparticles. Experimentally, a pulse Nd:YAG laser beam (40 mJ) was directed and focused by quartz lens (focal length of 30 mm) on a high purity Sn metal plate, which is immersed at the bottom of the petri dish in which contains liquid. Some liquid was used as media including pure water and ethylene glycol solution. Laser bombardment was made for 60 minutes in each liquid.

Characterization of tin oxide nanoparticles. Several tests were carried out to characterize the colloidal nanoparticles obtained. To determine the spectrum and absorbance level of tin oxide nanoparticles colloid (SnNPs), a UV-Visible Light Spectroscopy (UV-Vis) test was performed. The UV-Vis test was carried out by inserting 3-4 ml of colloidal SnNPs into the cuvette. The cuvette containing colloidal SnNPs was placed into the instrument and the data could be read after the UV-Vis instrument was operated. The Energy Dispersive X-ray (EDX) test was carried out to determine the atoms and compounds contained in colloidal SnNPs by dropping colloidal SnNPs nanoparticles on silicon carbide (SiC) plate measuring 0.5 cm x 0.5 cm and then dried at 30°C for 24 hours. The Fourier Transform Infra-Red (FTIR) Spectrophotometer test was also carried out to identify functional groups and compounds contained in SnNPs colloid. To see the morphology and size of tin oxide (SnO_2) nanoparticles, a Transmission Electron Microscope (TEM) test was performed. The image results from the TEM test were then processed using ImageJ software to determine the size distribution of the resulting tin oxide (SnO_2) nanoparticles (SnNPs) [8].

Antibacterial testing. Testing of SnNPs as an antibacterial agent was carried out using the disc diffusion method. The samples to be tested were based on variations in concentration using distilled water media with a concentration of 10 ppm, 20 ppm, and 30 ppm respectively. The disc diffusion method is carried out by measuring the diameter of the inhibition zone (DIZ), which is an indication of an inhibitory response to bacterial growth by an antibacterial compound in the extract. Before making the test medium, an important first step is to determine the number of bacteria in the media (disc). The bacterial suspension was diluted to have a turbidity level or optical density of 0.5 Mc Farland. The standard of 0.5 Mc Farland is the number of bacteria in 1 milliliter of suspension estimated at 1.5 x 10^8 bacteria. The test medium was made by mixing the agar nutrients with a diluted suspension of Escherichia coli bacteria. The media was stirred until evenly distributed then allowed to dry and solidify (plate medium). Furthermore, the filter paper that has been sterilized was then immersed in each colloidal SnNPs with various concentrations of 10 ppm, 20 ppm, and 30 ppm. The filter paper was then placed on the test media. The test media was put into an incubator at 37°C for 18-24 hours and the inhibition zone diameter was observed [9].

Results and Discussion

Effect of liquid medium on tin oxide nanoparticles (SnNPs). Synthesis results in pure water and 5 M ethylene glycol media produced brownish colored SnNPs as seen in Figs. 1(a) and 2(a), according to the results of the research of Torres-Mendieta et al [10]. After settling for one day, the colloidal color of SnNPs begins to fade to light brown. On the other hand, it is also found that very small particles are deposited at the bottom of the bottle as shown in Figs. 1(b) and 2(b). Two days after being synthesized, the colloidal color fades to become almost clear and it appears that more and more particles are found at the bottom of the bottle (Figs. 1(c) and 2(c)). Figures 1(d) and 2(d) are colloids that are left to stand six days after being synthesized; the color of the colloids becomes very clear and more and more particles are found at the bottom of the bottle. Particles produced from the bottom of the bottle are the result of agglomeration and precipitation of nanoparticles dispersed in a liquid medium.

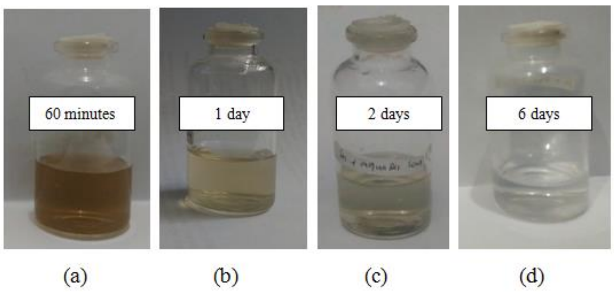


Fig. 1. Colloidal SnNPs in pure water.

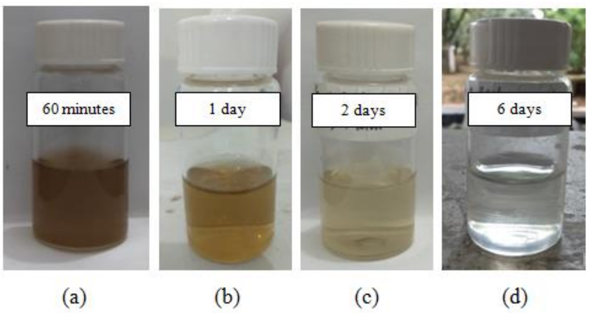


Fig. 2. Colloidal SnNPs in 5 M ethylene glycol medium.

It is hypothesized that shortly after the laser hits the target, atoms and ions are affixed from the surface of the target material to move randomly at high speed and produce high-temperature plasma. Atoms and ions move randomly so quickly that they collide with each other, the movement of atoms and ions is called Brownian motion. The hotter the temperature in the target material area, the higher the frequency of collisions between atoms/molecules and ions. The frequency of collisions between atoms and molecules increases, so the more the number of nanoparticles join together and agglomerated, and finally form larger NPs. Particles with a larger size that settles at the bottom of the bottle will make the concentration of NPs dispersed in the liquid media decreases (colloid becomes clearer) [11].

Analysis of Colloidal Absorption Spectrum SnNPs using UV-Vis. UV-Vis analysis was carried out to determine the intensity of the produced SnNPs colloids. Figure 3 shows the colloidal absorption spectrum of SnNPs in pure water and 5 M ethylene glycol medium.

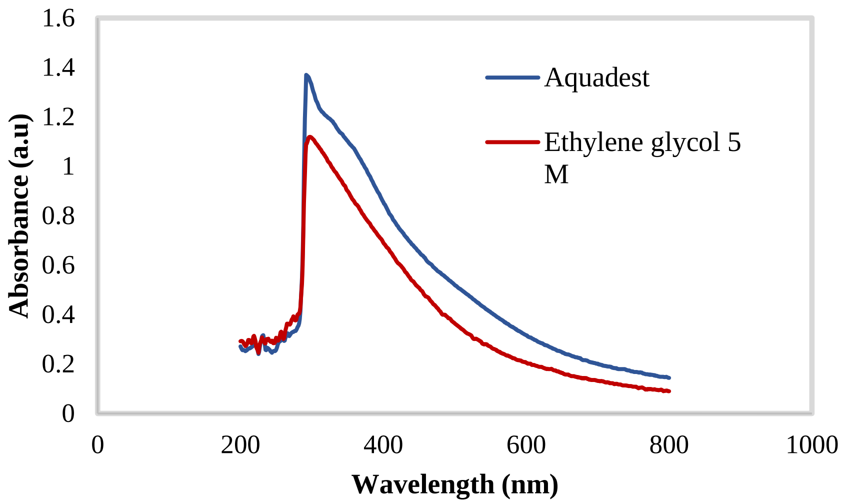
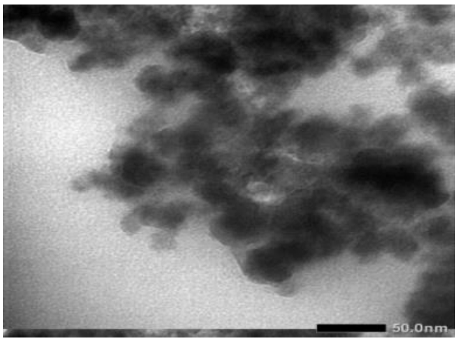


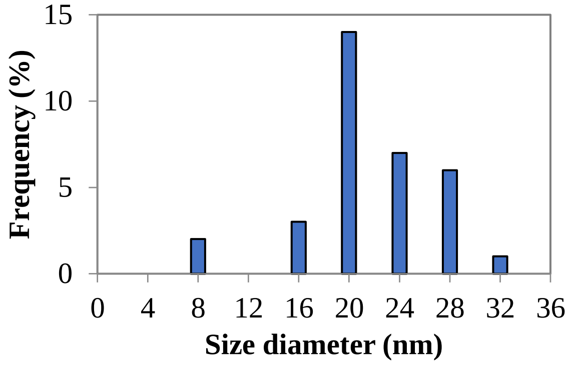
Fig. 3. The Colloidal absorption spectrum of SnNPs.

As shown in Fig. 3, both spectra have a single absorbance, where colloidal SnNPs in pure water media have a maximum absorbance of 1.37 a.u. while in the 5 M ethylene glycol medium, it has a maximum absorbance of 1.119 a.u. With the same duration of ablation and repetition rate at the time of synthesis, the 5 M ethylene glycol medium has a lower absorption intensity than in pure water. That is because the laser beam before hitting the surface of the Sn material target is absorbed by the ethylene glycol molecule and the laser energy that hits the target results in the target material being agglomerated being less so that the density of particles formed in the solution is reduced and makes the maximum absorption lower than SnNPs in pure water medium [12].

Morphological analysis of SnNPs. Figures 4 and 5 show TEM images along with the histogram size distribution of SnNPs synthesized using the pulse laser ablation method in pure water medium and 5 M ethylene glycol liquid media. The resulting TEM image shows the spherical shape of SnNPs, seen in Fig. 4 and 5 (a). From the figure, it can be seen that nanoparticles in pure water media are more clumped than in 5 M ethylene glycol medium, Fig. 5 (a). This shows that SnNPs in pure water media tend to be more aggregated and it is not easy to differentiate between one nanoparticle and another [13]. As a result of Brown's motion a particle moves quickly, so it tends to clot. Therefore, the final nature of nanofluids does not depend on the primary particles but the hydrodynamic size of the agglomeration of the formed nanoparticles [10]. Table 1 shows the averaged diameter of the SnNPs measured using ImageJ and their standard deviation.

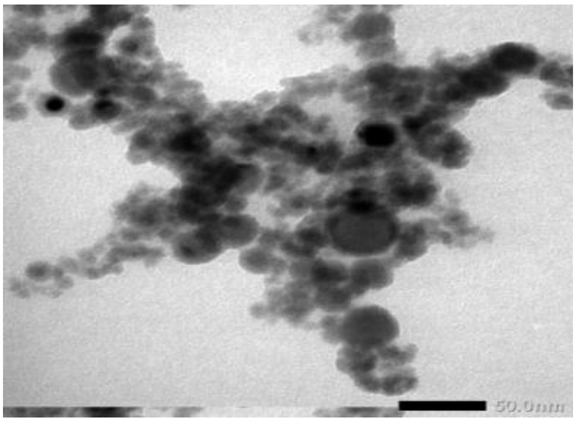


(a)

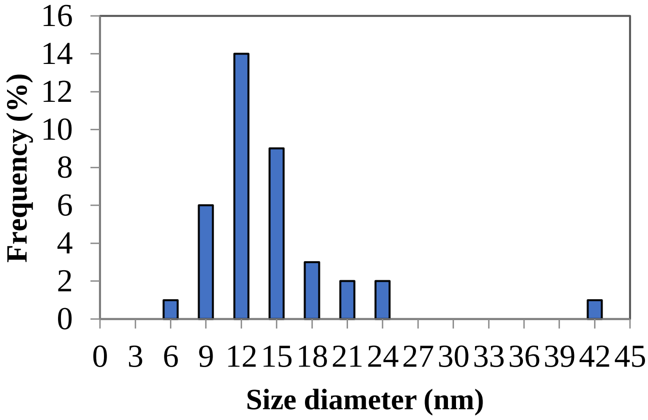


(b)

Fig. 4. (a) TEM image and (b) histogram SnNPs size in pure water medium.



(a)



(b)

Fig. 5 (a) TEM image and (b) histogram SnNPs size in 5 M ethylene glycol medium.

Table 1 The average diameter of tin oxide nanoparticles measured using ImageJ software based on TEM images

Sample	Liquid medium	Diameter average size (nm)	Standard deviation
1	Aquadest	19.98	5.00
2	Ethylene glycol	12.55	4.12

Adding ethylene glycol to pure water (which is a 5 M ethylene glycol solution) can reduce the average diameter of SnNPs from 19.98 nm to 12.55 nm. This decrease in the average diameter of the nanoparticles is because, in 5 M ethylene glycol liquid medium, the surface of the SnNPs is coated or blocked by ethylene glycol molecules, which make the nanoparticles scattered so well that it can help reduce the attachment of particles to each other, which results in the size of the nanoparticles becoming larger [13].

Identification of SnNPs colloidal. Tin oxide nanoparticles (SnNPs) can be identified by their elements and compounds using Electron Dispersive X-ray (EDX) and Fourier Transform Infra-Red (FTIR) devices. Figure 6 shows the EDX spectrum of colloidal SnNPs, while Table 2 is the percentage of elements and compounds produced from the EDX test.

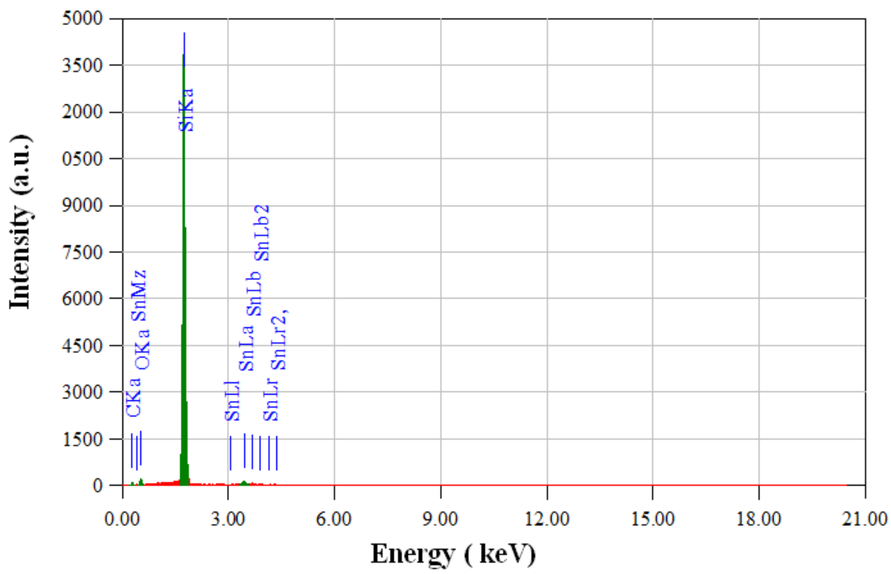


Fig. 6. EDX Spectrum of SnNPs

EDX analysis showed the produced colloidal SnNPs contain 41.17% silicon (Si), 1.61% tin (Sn), 47.34% oxygen (O), and 9.88% carbon (C) in the sample. Tin was detected in this spectrum because the material used for the manufacture of tin oxide (SnO_2) nanoparticles colloid is a tin plate with high purity (99.95%). Meanwhile, the presence of oxygen in the spectrum indicates the formation of tin oxide (SnO_2) compounds. The process of forming tin oxide (SnO_2) compounds occurs when the laser ablates the surface of the tin plate and forms a plasma. The water molecules on the surface of the tin plate will also be atomized and ionized due to the high temperature in the plasma area [14]. Oxygen resulting from the atomization of water molecules oxidizes the ablated tin from the surface of the tin plate, then forms tin oxide compounds. The silicon and carbon peaks in the spectrum come from the substrate used for this test, namely silicon carbide (SiC) plates. It also shows that the colloidal tin oxide (SnO_2) nanoparticles made in this study have high purity because there are no foreign elements found in the EDX spectrum.

Table 2. Percentage of elements and compounds produced from the EDX test.

Element	Mass (%)	Compound	Mass (%)
C	9.88	C	9.88
O	47.34		
Si	41.17	SiO_2	88.08
Sn	1.61	SnO_2	2.04

FTIR analysis is also made to identify compounds of SnO_2 (SnNPs). Figure 7 shows the FTIR spectrum of colloidal SnNPs synthesis. The absorption peaks at 3393.32 cm^{-1} and 1643.32 cm^{-1} were detected from pure water liquid medium hydroxyl groups. Bands that appear in the range 400-700 cm^{-1} are Sn-O anti-symmetric characteristics. The presence of the 629.03 cm^{-1} peak in the synthesized sample confirms the presence of SnO_2 [15]. The peak of transmittance between 600-660 cm^{-1} is characteristic of SnO_2 [16].

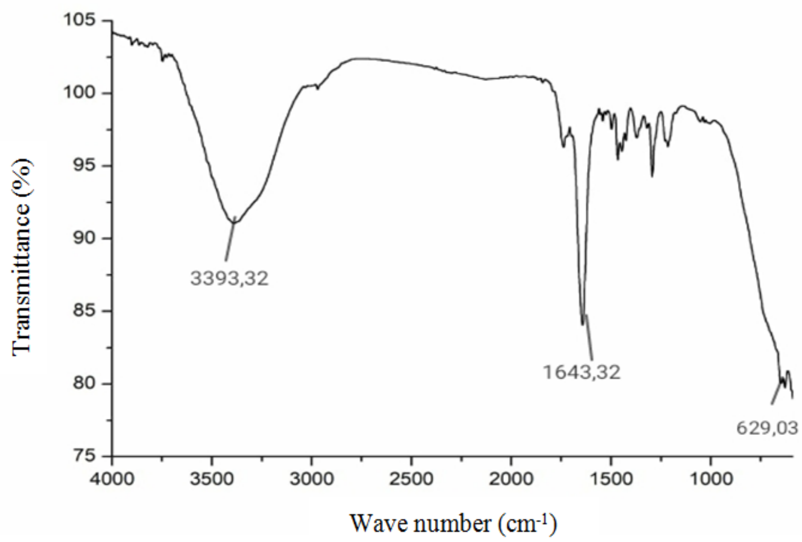


Fig. 7. SnNPs FTIR spectrum.

Antibacterial activity of E. coli. Antibacterial activity test using the disk diffusion method was carried out by measuring the diameter of the inhibition zone (DIZ), which is an indication of the response to inhibiting bacterial growth by an antibacterial compound in the extract [9]. Positive control media using Terramycin, which is an antibiotic and contains strong antimicrobial effects. Terramycin is a versatile and effective antibiotic to treat infections caused by gram-positive and gram-negative bacteria such as E. coli. While the negative control media using a Dimethyl Sulfoxide (DMSO) solution which has no activity against the test bacteria.

The results of the antibacterial activity test using the disk diffusion method in Table 2 shows that the negative control with DIZ 0 mm, shows that the negative control using DMSO is resistant, which means the bacteria cannot be killed. A positive control using Terramycin on E. coli bacteria showed a pretty good antibacterial effect, DIZ was quite large at 38 mm, which indicates that there was an inhibition of bacterial and bacterial activity in the death phase.

Table 3. Test results for E. coli bacteria activity.

No	Sample	Inhibition zone diameter (mm)
1	Negative control	0
2	Positive control	38.00
3	10 ppm	6.50
4	20 ppm	6.75
5	30 ppm	9.50

Colloidal SnNPs against the bacteria tested, the largest DIZ occurred in colloidal SnNPs with a concentration of 30 ppm, the next 20 ppm, and the smallest DIZ at a concentration of 10 ppm. This shows that the higher the concentration of SnNPs, the inhibition of bacterial activity will also increase [17].

Conclusion

Based on the results obtained in this study, several conclusions can be formulated including the synthesis of SnNPs using the PLA Nd: YAG method successfully carried out in pure water and 5 M ethylene glycol medium with a repetition rate of 10 Hz resulting in brownish colloidal SnNPs. UV-Vis characterization produced absorbance values in pure water and 5 M ethylene glycol mediums of 1.314 a.u. and 1.119 a.u., respectively. The results of EDX and FTIR characterization showed that the synthesized SnNPs colloids consisted of SnO_2 compounds. The morphology of SnNPs by TEM images in 5 M ethylene glycol medium is spherical with an average diameter of 12.55 nm, which is smaller than the diameter size in a pure water medium, which is that of 19.98 nm. The results of antibacterial activity tests using the disk diffusion method showed the largest DIZ sequentially at SnNPs concentrations of 30 ppm, 20 ppm, and 10 ppm were 9.5 mm, 6.75 mm, and 6.50 mm.

Acknowledgment

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6. Submission kedua setelah proses review (30 Desember 2020)

webmail FISIKA universitas diponegoro

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

JNanoR

Ali Khumaeni <khumaeni@fisika.fsm.undip.ac.id> Thu, Dec 31, 2020 at 12:20 PM
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Best regards
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7. **Acceptance letter dari jurnal dari seluruh proses review (3 Maret 2021)**

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1 message

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9. Notifikasi publikasi dari Journal of Nano Research (30 Juli 2021)

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