

Synthesis of Tin Oxide Nanoparticles by Pulsed Laser Ablation Method Using Low-Energy Nd YAG Laser as an Antibacterial Agent

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Synthesis of Tin Oxide Nanoparticles by Pulsed Laser Ablation Method Using Low-Energy Nd: YAG Laser as an Antibacterial Agent

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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].

¹²Bacteria 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 1.5×10^8 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.

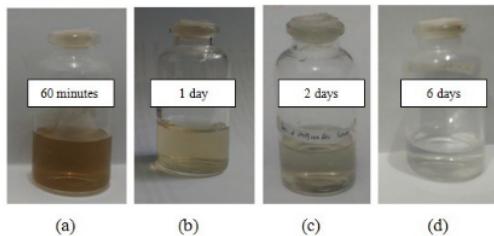


Fig. 1. Colloidal SnNPs in pure water.

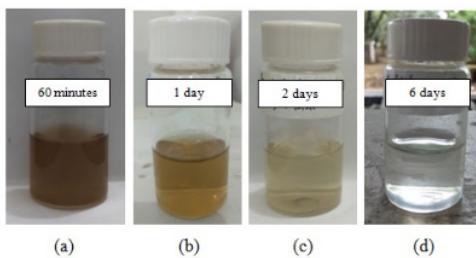


Fig. 2. Colloidal SnNPs in 5 M ethylene glycol medium.

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.

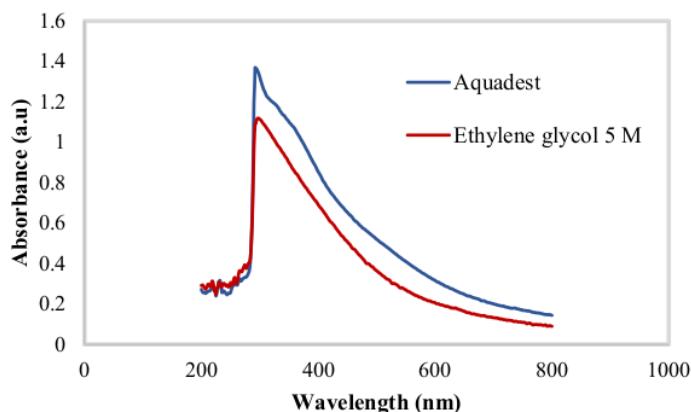
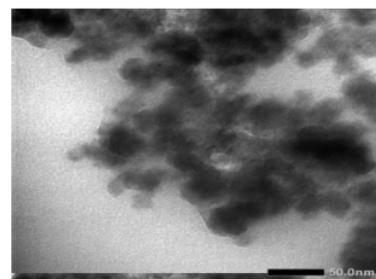


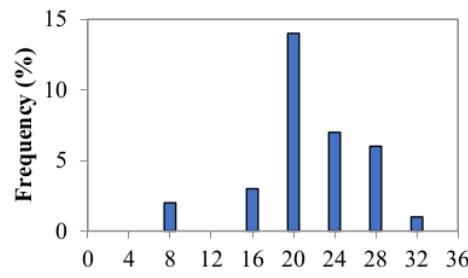
Fig. 3. The Colloidal absorption spectrum of SnNPs.

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.

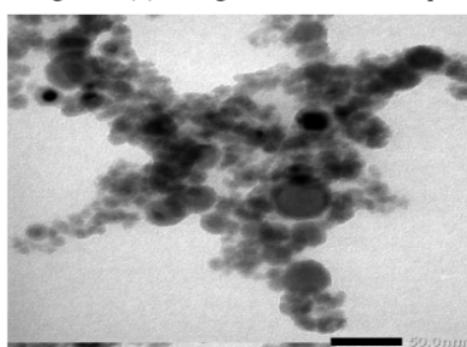


(a)

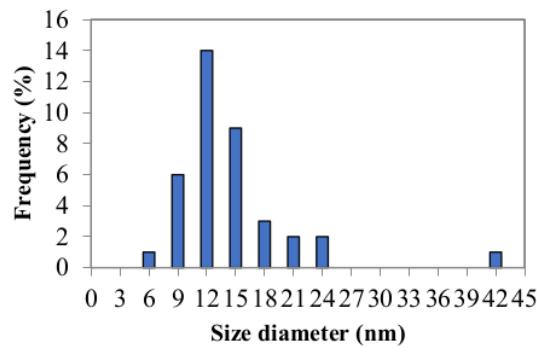


(b)

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.

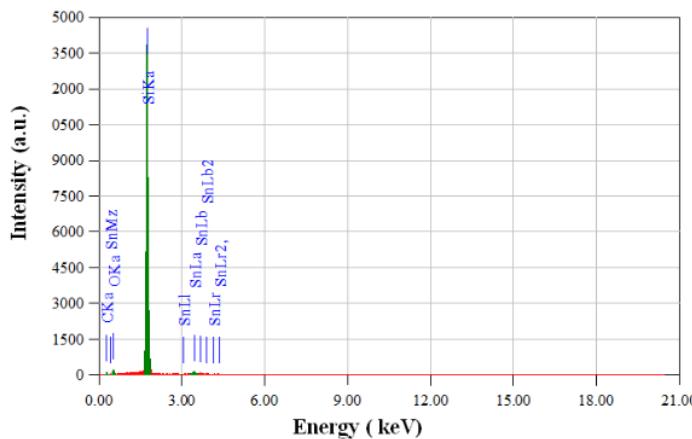


Fig. 6. EDX Spectrum of SnNPs.

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 SnO₂ (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 SnO₂ [15]. The peak of transmittance between 600-660 cm⁻¹ is characteristic of SnO₂ [16].

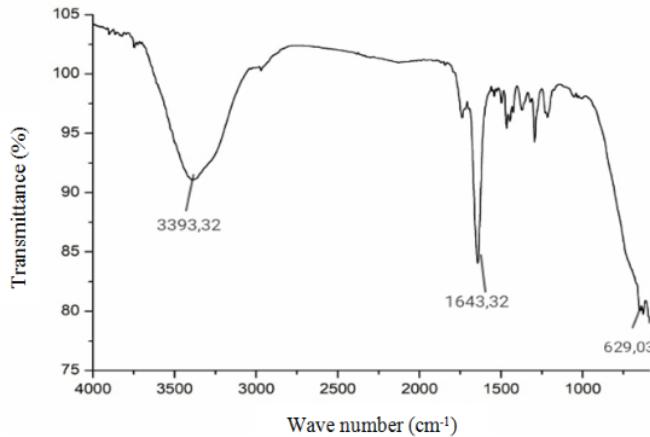


Fig. 7. SnNPs FTIR spectrum.

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.

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