Antibacterial activity of ZnO nanoparticles fabricated using laser ablation in solution technique

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Abstract. The zinc oxide (ZnO) nanoparticles have been produced by using laser ablation in solution technique, resulting nanoparticles with hexagonal wurtzite structure and the size range of 20–50 nm. The optical characterization has predicted the nanoparticles band gap at least 3.16 eV. The ZnO nanoparticles were employed as an antibacterial agent for the growth of Escherichia coli (E. coli), and it shows a great potential. Total plate count (TPC) analysis was conducted by applying the ZnO nanoparticles with different concentration of 5%, 10%, 15%, 20%, 25% and 30% (portion of bacteria medium). The 5% concentration could reduce more than half of the E. coli population after 24 hours incubation time. There was no living bacteria detected for 20%, 25% and 30% ZnO concentration.

1. Introduction
The growth of research on zinc oxide for antibacterial agent has been exceeding greatly in the past decade. Some of research work has showed the promising result of ZnO as bacterial agent as reported by [1-4]. Indicating ZnO as a great potential antibacterial agent for gram positive bacteria (Escherichia coli) and gram negative bacteria (Staphylococcus aureus) [5-7]. The bactericidal activities ZnO is more effective when the size of ZnO particles are reduced to a nano-scale, however the ZnO nanoparticles (ZnO-NPs) are also known to be non-toxic and biocompatible with human cells [8, 9]. The generation of reactive oxygen species (ROS) and release of Zn2+ are two antibacterial mechanisms of ZnO-NPs which have been proposed because these processes can destroy the membrane of the bacteria [7]. Future modification of ZnO-NPs is very feasible by structure modification and combining ZnO with other chemical elements to increase antibacterial activity [10,11].

Laser ablation in liquid technique is one effective method to produce ZnO nanoparticles. Some previous works have applied this method to produce ZnO-NPs in pure water [12-14]. Janice low et.al has performed the synthesis of ZnO-NPs using laser ablation technique resulting ZnO-NPs with the range size of 100-150 nm [15]. They also conducted antibacterial test by observing disk-diffusion agar method. However, it showed no significant result on bactericidal activity of the produced ZnO-NPs where it might be due low concentration of ZnO-NPs [15].

This paper presents our work on ZnO-NPs production using laser ablation technique in pure water for antibacterial application. The produced ZnO-NPs will be first characterized for its optical and structure properties. We employed TEM and XRD to study the morphology and crystal structure of...
the nanoparticles. The UV-visible spectrophotometer was used to know the optical properties and band gap energy of the produced ZnO-NPs. In this work, the effectiveness of ZnO-NPs as the antibacterial agent will be studied by observing its optical density and bacteria population reduction. For this purpose, some variation of ZnO-NPs concentration, 5%, 10%, 15%, 20%, 25% and 30%, was applied to the bacteria medium. The remained population will be counted by total plate count (TPC). Escherichia coli (E. coli) was chosen as the tested bacteria because it is the most common bacteria found near human activity and needs, such as food and water.

2. Methodology

2.1 Synthesis of ZnO nanoparticles by laser ablation in solution technique

The schematic diagram of this experiment is shown in figure 1. The laser ablation in solution technique consists of Quanta-Ray Spectra-Physics Nd-YAG laser with the wavelength of 1064 nm, energy of 105 mJ and frequency of 10 Hz. The laser beam was focused into a zinc metal target with the purity of 99.5% (purchased from Nilaco corp Japan). The 150 mm focal length plano-convex lens was used for the focusing purpose. The zinc plat was attached inside a beaker glass and the glass was placed on top of rotation stage. For the solution, we used 5 mL of pure water and its height was about 7 mm from the top of zinc plate. The weight of zinc plate was measured before and after ablation to predict the concentration of ZnO-NPs in the colloid. The ablation process took place for 60 minutes.

![Figure 1. Experiment set-up of laser ablation in pure water for ZnO-NPs fabrication](image)

2.2 Structure and optical characterization of ZnO nanoparticles

From the laser ablation process we have obtained around 5 mL milky-colour ZnO-NPs colloid. This sample was prepared for structure and optical properties characterization before it was used for antibacterial testing. The transmission electron microscope (TEM) was employed to see the structure of ZnO-NPs. X-ray diffraction measurement unit was used to study the crystallinity our produced ZnO-NPs. The colloid sample was put in petri dish and it was dried inside an oven with temperature of 40°C. From this process we obtained ZnO-NPs powder for XRD characterization. For optical analysis, we measured the absorbance of our nanoparticles on UV and visible region by using UV-vis spectrophotometer. The photoluminescence analysis was conducted by employing 325 femtosecond
laser and MAYA spectrograph according to our home-made PL set-up in laser lab research center for physics.

2.3 Antibacterial testing

Antibacterial testing was conducted using two methods, optical density analysis and total plate counting. We chose E. coli for our analysis because it was the most common bacteria which was found near human needs and activity. The bacteria were growth in nutrient broth (NB) as the medium, and it was incubated for about 24 hours in 37°C environment. The ZnO-NPs colloid obtained from ablation process was used for antibacterial agent, and it was prepared with six different, sample 2 - sample 3 concentrations as mentioned in table 1. Sample 1 is a control sample which is consisted only bacteria and its medium, without ZnO-NPs. The concentration mentioned in the table portions of ZnO colloids inside the total medium and bacteria. The total volume of medium and bacteria used in the sample glass is 2500 μL. Hence 5% of ZnO-NPs concentration means we put 125 μL ZnO-NPs colloid inside 2500 μL of observed bacteria and medium.

| Sample name     | ZnO-NPs concentration (%) | ZnO-NPs concentration (μL) |
|-----------------|---------------------------|----------------------------|
| Sample 1 (control) | 0                         | 0                          |
| Sample 2        | 5                         | 125                        |
| Sample 3        | 10                        | 250                        |
| Sample 4        | 15                        | 375                        |
| Sample 5        | 20                        | 500                        |
| Sample 6        | 25                        | 625                        |
| Sample 7        | 30                        | 750                        |

Optical density was measured using spectrograph with the wavelength of source light is 600 nm. Afterwards, we used total plate counting methods to calculate the growth bacteria after ZnO-NPs were applied as antibacterial agent. Both analyses were conducted after 24 hours of incubation time.

3. Result and discussion

3.1 The structure of ZnO nanoparticles
Figure 2. TEM image of ZnO-NPs produced by laser ablation in pure water

After an hour ablation process, ZnO colloid sample was obtained and it was predicted to have around 0.5 mg/mL of ZnO-NPs concentration in water. From TEM imaged on fig. 2, it was observed that the ZnO-NPs were formed after the laser ablation process in the liquid. The shape of nanoparticles tends to be rod shaped. However, some of spherical shape was still found on the TEM image, indicating a non-uniform shape. The size was measured to be in the range of 20 – 100 nm with most of the grain to have size around 50 nm. This result shows a good agreement with some previous work on producing ZnO-NPs using laser ablation technique in water [15, 16]. The rising of temperature due to ablation process can be one reason of the elongation of the grain. The absence of surfactant also increases the chance of agglomeration of nanoparticles.

The XRD spectrum is presented at fig. 3. ZnO-NPs prepared in pure water exhibits strong diffraction peaks with the index of (1 0 0), (0 0 2), (1 0 1), (1 0 2), (1 1 0), (1 0 3), (2 0 0), (1 1 2) and (2 0 1). All peaks were indexed as the hexagonal wurtzite structure of ZnO. There is no other phases were observed, indicating the absence of metallic Zn inclusions and the strong aqueous oxidation has perfectly occurred on converting Zn into ZnO. Crystallite size along (100), (002), (101) crystallographic plane for this sample, as calculated by Debye Scherer formula, are respectively defined to be 20.31, 29.36 and 19.70 nm. The calculated crystallite size shows an acceptable result and it is smaller than the nanoparticles size showed on the TEM image.
3.2 The optical properties of ZnO nanoparticles

Fig. 4(a) and 4(b) shows the UV - visible light absorbance and its $h\nu$ plot to predict the band gap energy of the produced ZnO nanoparticles. The absorbance shows the single peak of ZnO at around 341.89 nm which agreed well with the XRD spectra discussed above. The absorbance spectra was converted into a Tauc plot following, $E = h \times c \times \lambda^{-1}$ formula. A Tauc plot is used to determine the optical bandgap, or Tauc gap, for semiconductor materials. Fig. 4(b) shows predicted band gap energy at around 3.16 eV. This value matches very well as some previous study on ZnO nanoparticles synthesis and its bandgap determination [1-5]. The typical photoluminescence spectra of ZnO produced in in pure water is presented in Fig. 5. The spectra exhibited an UV emission band around 3.13 eV (396.5 nm). The emission in the UV region is attributed to the recombination between electrons in the conduction band and the holes in the valence band (exciting emission). The green emission at 2.26 eV (548.3 nm) is related to the defects and impurities of our produced ZnO-NPs in water solution.
3.3 Antibacterial activity of ZnO nanoparticles

The target bacteria, E. Coli, were grown in nutrient broth at 37°C for 24 hours aerobically. Bacterial growth was determined by absorption or optical density measured at 600 nm after 24 hours incubation we obtained the optical density values as presented on table 2. In the absence of ZnO nanoparticles of sample 1, the optical density recorded was maximum indicating an intense growth of bacteria strain in nutrient broth medium. The growth of bacteria was able to be inhibited by applying ZnO-NPS starting from 5% concentration. It reaches 50% reduction of bacteria growth as we used high amount of ZnO-NPs, 30% portion of medium.

| Sample name   | ZnO-NPs concentration (%) | ZnO-NPs concentration (μL) | OD (au) |
|---------------|----------------------------|----------------------------|---------|
| Sample 1 (control) | 0                          | 0                          | 1,244   |
| Sample 2      | 5                          | 125                        | 0,961   |
| Sample 3      | 10                         | 250                        | 0,804   |
| Sample 4      | 15                         | 375                        | 0,901   |
| Sample 5      | 20                         | 500                        | 0,855   |
| Sample 6      | 25                         | 625                        | 0,753   |
| Sample 7      | 30                         | 750                        | 0,654   |

Total plate counting method was used for calculating the bacteria population. The data were presented on Fig. 6. The ZnO-NPs concentration was varied same as optical density analysis. After 24 hours treatment, control sample (sample 1) shows great number cfu/mL (colony-forming units per
millilitre) of E. coli population. It reaches $1.46 \times 10^8 \text{ cfu/mL}$. The bacteria population has reduced significantly by applying 5% of ZnO-NPs. There are no living bacteria found as we applied 20%, 25% and 30%. The antimicrobial activity of ZnO has enhanced due to the presence of water molecules. The aqueous suspensions of ZnO and water lead to the generation of free radicals (hydroxyl and oxygen species) which is responsible for strong oxidative stress in the bacterial cells. Our result shows greater bactericidal activity as we increase the ZnO-NPs concentration and we suspect the enhancement of free radicals generation as the main reason.

![Figure 6. Photoluminescence of ZnO nanoparticles produced by laser ablation in pure water](image)

4. Conclusion

Zinc oxide nanoparticles are able to be produced by using laser ablation in pure water. The shape of nanoparticles tends to be rod shaped with the size range of 20 – 100 nm. The XRD spectra show all peaks were indexed as the hexagonal wurtzite structure of ZnO, with the crystallite size is calculated to be 20.31, 29.36 and 19.70 nm for three major peaks. The optical analysis by observing UV-vis spectra show a single ZnO peak at around 341.89 nm and a bandgap energy at around 3.16 eV which have good agreement with some previous work. As we applied our produced ZnO-NPs as antibacterial agent on E. coli growth, we saw a great potential from our result. After 24 hours bacteria incubation time, it shows that the higher concentration of ZnO-NPs (over the total medium and bacteria) the higher bactericidal activity is obtained in this analysis. Optical density values decrease significantly by applying 5% up to 30% ZnO-NPs concentration. For quantitative analysis using total plate counting, more than 50% of total E. coli population is able to be reduced by using the minimum concentration of ZnO-NPs. There is no living bacteria found as we used 20%, 25% and 30% concentration of ZnO-NPs.

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