Antibacterial activity of Zinc Oxide nanostructured materials synthesis by laser ablation method

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Abstract. Zinc oxide (ZnO) nanostructure (NS) materials with different shapes were synthesis via pulsed laser ablation in water. The characterizations were done using X-ray diffraction XRD, and scanning electron microscopy SEM. The XRD results proved the presence of the (100) and (002) patterns, referring to the ZnO NS. The SEM images show that the structures were changed from flakes with a thickness of about 10-50 nm to spherical like structures with diameter from 24 -42 nm and high agglomerated. Also, the antibacterial activity of ZnO NS was studied and the results manifested that the inhibition zone in Staphylococcus aureus (S.aureus) is higher than in Escherichia coli (E.coli), which showed an inhibition zone against S. aureus (21mm), as well as against E.coli was (15mm) for ZnO NS prepared at 25 laser pulses, and these activities increased with an increased number of laser pulses for both type of bacteria. Therefore the ZnO NS materials are recommended as a powerful Anti-bacterial material

Keywords: Nanostructure; Laser ablation in liquid; ZnO NS; antibacterial activity

1. Introduction
Metal oxides nanostructured materials are very interesting material in many types of research owing to their unique properties and useful applicability in various disciplines for photodetector, catalysis, Solar cell, and biomedical fields [1-6]. ZnO is one of the powerful nanostructure materials that can be used in different areas of science and technology due to novel features such as high binding energy, non-toxic, and large bandgap energy. It was established to initiate in assorted applications such as UV photodetectors [7], solar cells [8], sensors [9], varistors [10], and antibacterial agent [11]. In general, ZnO nanostructure material is the main metal oxide nanomaterials that been used as an antibacterial agent owing to its features of a mineral that is necessary to humans, non-toxic, stable, and more active even at lower concentrations uses [12]. These materials can be prepared by different methods like chemical vapor deposition (CVD) [13], sol-gel [14], a chemical method [15], spray pyrolysis [8], hydrothermal process [16], and co-precipitation method [17]. Among these, Pulse laser ablation in liquid (PLAL) process is a new track that used to prepare ZnO nanostructure material owing to their benefit such as uncontaminated, straightforward, and extraordinary pure manufacture [18, 19]. In addition, this method possibly will be applied to produce special kinds of nanomaterials with an unusual morphology that can be controlled by adjustment of the laser process features such as...
wavelength, energy, pulse duration, and the number of laser pulses, also the liquid type and its height over the plate effected on the properties of prepared nanomaterial[20-23]. In this research, a ZnO NPS synthesized via Nd: YAG laser ablation of zinc plate in water and studied their antibacterial activities against two species of bacteria.

2. Experimental work
2.1. Preparation of ZnO NS
Q-switching Nd-YAG laser (1064 nm; 9 ns; 1 Hz) irradiate system was used to ablate the zinc target (99.9%) impressed in 3ml of water containing a small glass vessel with continuous spin to avoid the target agent by simple stirrer. The ablation process was employed with laser energy about 700mJ and changed the number of laser pulses from 25 – 125 pulse.

2.2. Characterization of ZnO NS
The characterization of these nanoparticles were measured using Philips PW (Japan) X- Ray diffraction pattern (XRD) with Cu-Kα radiance source at 2θ angle = (25 - 45 ), and scan speed 5.0000 (deg/min), and the morphology was recognized via Scanning electron microscopy ( MERA3 TE) operating with 10KeV

2.3. Antibacterial activity of ZnO NS
The antibacterial activity of ZnO NS against two bacteria is E. coli (gram-negative) and S. aureus (gram-positive) was estimated using the agar method, for more details please see reference [11]. The diameters of wells were 6mm, and the nanoparticles were added with different concentrations, then the plates were an incubator for 24 h. The activity was established by assessing the diameters of the inhibition zone (IZ).

3. Result and Discussion
XRD results for ZnO NS that presented in Figure 1. indicated the sample pattern which indexed as the hexagonal wurtzite structure. Three peaks can be detected at 20 ≈ 31°, 34°, and 36° indexed as (100), (002), and (101). The XRD patterns of all samples were in good agreement standard card 36-1451, and data in references [24-26].

![Figure 1 XRD for ZnO NS synthesis with different laser pulses](image)

Figure 2 showed an SEM view of ZnO NS produced via pulsed laser ablation at 700mJ and for different laser pulses (50, 75, 100, & 125). One can see that the morphology of prepared ZnO NS was a change with laser pulses, which look like flakes with a thickness of about 10-50 nm at 50 pulses, while it change to particles with spherical like structures at 75 pulses, with diameter from 24 - 42 nm.
At 100 laser pulses, the particles were mostly spherical shape with high aggregation. Finally, in 125 pulses, tiny aggregations of spherical particles were observed. Figure 3 clarifies the inhibition zone pictures of ZnO NS for two bacteria. In general, it was observed that the inhibition (IZ) in Gram-positive bacteria (S.aureus) is larger than in Gram-negative bacteria (E.coli), which found that the diameters of IZ for E.coli were treated with ZnO NS were 15, 22, 20, 27, and 34 mm and the diameters for S.aureus were 21,25,27,30, and 32 mm. Also, shows that the inhibition zone increased as an increased number of laser pulses for both types of bacteria this may be owing to increasing the number of particles or particles concentration in the solution. In general, these findings have shown that S.aureus is highly sensitive to ZnO NS than E.coli. This may be because of the various susceptibilities characteristic of each bacterial species to ZnO NS comes from the differences in the structure of the wall [27].

![SEM image for ZnO NS prepared with different pulses.](image-url)

Figure 2 SEM image for ZnO NS prepared with different pulses.
The activity mechanism of nanoparticle occurs after two stages. The first stage is by making damage in the outer membrane of the cell by electrostatic interaction between the wall and the NS. The second stage by producing the active oxygen environment, which is named oxidative stress like \( \text{H}_2\text{O}_2 \); this is because of the presence of zinc oxide NS affected on \( E. \text{coli} \). There is no change in the cell shape but when ZnO NS adheres to the cell surface is causing to generate irregular cell surface, membrane bleeding, outer membrane exhaustion, which causes leakage. Thus, the duty of NS as bactericidal or as a bacteriostatic agent, in other words, the activation of ZnO NS on bacteria is summarized by the direct interaction that affects the permeability of the outer membrane of bacteria. This causes oxidative stress in bacteria cell and effect on the inhibition development leading to bacteria death [28].

4. Conclusion
Pulse laser ablation in liquid method was the best and simple process to synthesis ZnO NS. The XRD results proved the formation of ZnO NS at \( 2\theta \approx 31^\circ, 34^\circ, \) and \( 36^\circ \) which indexed as \( (100), (002), \) and \( (101) \). SEM images show the structure was changed from flakes to spherical like structures with high agglomerated as laser pulses increased. Finally, the antibacterial activity results manifested that the inhibition zone in \( S.\text{aureus} \) is higher than in \( E.\text{coli} \), which show an inhibition zone against \( S.\text{aureus} \) was 21mm, while it was 15mm against \( E.\text{coli} \) for ZnO NS prepared at 25 laser pulses, and these activities increased with an increased number of laser pulses for both type of bacteria, therefore the ZnO NS materials are recommended as a powerful Anti-bacterial material.
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