



Synthesis of High-Performance Antibacterial Magnesium Oxide Nanostructures through Laser Ablation

¹Sara Fadhil Abbas*, ²Adawiya J. Haider, ³Sharafaldin Al-Musawi, ⁴Bakr Ahmed Taha

¹Department of Laser and Optoelectronics Engineering, College of Engineering, Al-Nahrain University – Iraq

²Laser Science and Technology Branch, Department of Applied Sciences, University of Technology – Iraq

³College of Food Sciences, Al-Qasim Green University – Iraq

⁴Department of Electrical, Electronic and Systems Engineering, Universiti Kebangsaan – Malaysia

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*Corresponding Author:

Sara Fadhil Abbas

sara.fadhil@nahrainuniv.edu.iq

Abstract

In this study, we synthesized magnesium oxide (MgO) nano flakes (NFs) through pulsed laser ablation of magnesium ribbons, investigating their potent antibacterial properties for potential biomedical applications. Thorough characterization utilizing advanced analytical techniques verified the phase purity and functionality of the fabricated MgO NFs. Results revealed a distinctive flake-like structure with an average diameter of 100–400 nm and a slender wall thickness of 24 nm. The efficiency of the laser ablation method was validated by EDX imaging, showing high purity in the MgO sample. XRD analysis further confirmed the polycrystalline nature of MgO NFs, with dominant peaks at 2θ values of 38.86° , 59.46° , 62.83° , and 73.87° corresponding to (111), (110), (220), and (311) diffractions, respectively. UV-visible spectroscopy exhibited a broad absorption peak, and Tauc's formula yielded an energy band gap of 5.8 eV. FTIR spectroscopy detected Mg–O–Mg bending vibration, O–H stretching vibration, O=C=O stretching, and O–H bending vibration. Optimized MgO-NFs demonstrated remarkable antibacterial efficacy against both gram-positive *Staphylococcus aureus* (*S. aureus*) and gram-negative *Escherichia coli* (*E. coli*) bacteria. Maximum antibacterial activity was observed at a high MgO NFs concentration (200 $\mu\text{g/ml}$), resulting in 15 mm \pm 0.5 mm and 16 mm \pm 0.5 mm inhibition zones for *E. coli* and *S. aureus*, respectively. The minimum inhibitory concentration (MIC) for both pathogens was determined to be 25 $\mu\text{g/ml}$, emphasizing the promising antimicrobial potential of the MgO NFs.

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1. Introduction

Science is currently focused on nanotechnology owing to the wide range of new possibilities in numerous fields of agriculture, food processing, and medicine [1]. The shape and size of colloidal metal nanoparticles play a

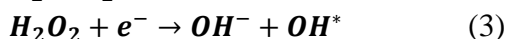
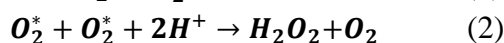
significant part in several applications [2]. These applications include synthesizing magnetic material, electronic devices, wound healing, antimicrobial, and bio-composites preparation [3]. Nanoparticle (NP) materials are of fair interest for various applications and studies because of their size-dependent properties. Researchers have given several types of nanoparticles and their derivatives, like nano-metals, high attention because of their potential antibacterial effects [4]. Inorganic nanoparticles, including metals and metal oxides, possess great antibacterial properties and might be alternatives to antibiotics for treating infection [5].

Infection happens after viruses, bacteria, or other microorganisms enter the body and reproduce [6, 7]. One of the most serious issues confronting health systems today is antimicrobial resistance (AMR), which happens when microbes that cause infection are in contact with a medicine that would ordinarily destroy them or prevent their development (i.e., antibiotics) [8]. AMR is caused by bacteria's ability to evolve and adapt to specific treatments [9, 10]. Besides this issue, antibiotics can also have negative consequences on the host, such as allergic responses, immunosuppression, and hypersensitivity [11, 12]. New techniques rely on metal oxide nanoparticles to diversify treatments to prevent the development of resistances as much as possible [13].

Magnesium oxide (MgO) nanoparticles are among the essential minerals for human health [14]. MgO attracted increasing attention in tissue engineering and regenerative medicine applications due to their biocompatibility, biodegradability, and bioactivity [15]. MgO is an insulator/semiconductor typically with a cubic structure [16]. Because of its relatively large band gap of 7.8 eV, MgO has limited applications. MgO NPs have a lower band gap in the range of 5 eV [17]. MgO at the nanoscale has a broad spectrum of bactericidal effects on both bacterial gram strains. It has reduced toxicity and more mammalian bioactivity compared to most metal oxides.

As a result, it might be used as an ingredient in medication composition [18]. Because there are so many uses for MgO nanoparticles and nanocomposite, many publications on their synthesis have been published. Sawai et al. found that MgO exhibited a strong capacity for inhibiting bacteria [19].

The process by which magnesium oxide nanoparticle inhibited bacteria is still not clear. This mechanism may related to oxygen reacts with the surface of bacteria to produce superoxide radicals gives rise to antibacterial activity. The high reactivity of the additional electrons may harm proteins and phospholipids in the bacterial membrane [19, 20]. MgO NPs are effective in creating reactive oxygen species (ROS) that are important for their antibacterial activity, including hydrogen peroxide (H_2O_2), hydroxyl radicals ($\cdot OH$), and superoxide radicals (O_2^-) hence these radicals also cause rupture of the cell membrane [21-24] the chemical formula represent the production of ROS[25]:



MgO NPs also suppress cells by releasing harmful ions (Mg^{2+}) when they enter cells. When these harmful ions come into contact with amino acids (thiol-groups), proteins' regular structures are destroyed, ultimately resulting in cell death [26, 27]. MgO NPs also have an additional inhibitory mechanism that stems from their ability to impede essential enzyme functions in microorganisms. They can impede or slow down the growth of cells by, for example, inhibiting enzymes required for cellular respiration and energy synthesis, which can upset metabolic processes [28]. The whole proposed mechanism illustrated in Fig. 1.

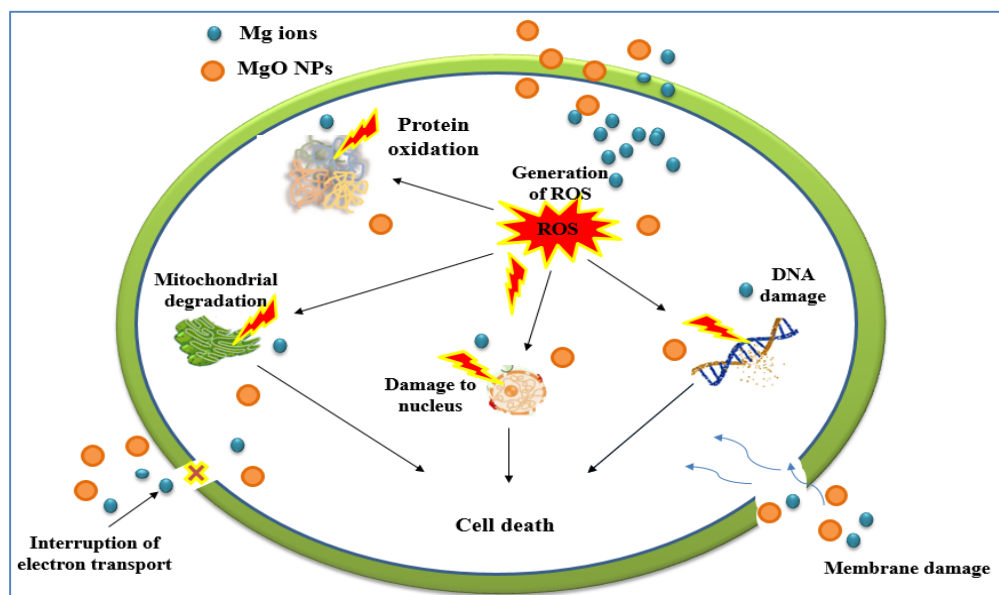


Figure 1: Proposed mechanism of MgO NPs antibacterial action

The antimicrobial mechanism of MgO NPs is a complex process and can be affected by various parameters. Prior findings demonstrated that the antibacterial action of MgO NPs is contingent upon the bacterial strain's specific shape, nature, and size [29, 30]. Marsili et al. described the antibacterial activity of zinc oxide NPs depending on the form of the particles [31]. Though the most prevalent shape of NPs is spherical [10], nano-cubes and nanorods are more effective than other shapes because of their exposed planes and metal oxidation levels [32, 33]. It has been proposed that the ability to kill bacteria of the NPs is correlated with the stability of the planes, with less stable planes and energy required to generate oxygen vacancies [34]. Wang et al. showed that because of their exposed crystal surfaces and capacity for oxidation, nanorods exhibited more bactericidal activity than other forms [35].

Many different preparation paths provide MgO NPs containing sol–gel [36], hydrothermal/solvothermal [37], chemical gas phase deposition [38], aqueous wet chemical method [39], hydrothermal method [40], and so on. Using laser ablation method could overcome many restrictions, such as the requirement of advanced tools, numerous processing stages, additional time, surfactant, template, etc. It delivers essential advantages for biological applications of creating NPs without additional impurities, and the processing setup prices, are not high [33]. The success of the laser-assisted pulsed approach in creating various sizes and forms of nanoparticles, which are employed in many applications, makes it significant in a liquid environment [41, 42]. The method's greatest distinguishing characteristic is the non-equilibrium growth process, brought on by plasma created by laser ablation of a sample in solution. It has exceptionally elevated pressure and temperature [43]. In the present study MgO nano flakes synthesize using a pulsed laser ablation in liquid method. Later, MgO nano flakes characterized their optical, structural, chemical, morphological, compositional, surface charge and particle size. Studying MgO nano flakes effect of antibacterial activity against different tested bacterial' strains gram-positive *S. aureus* and gram-negative *E. coli* bacteria.

2. Experimental Procedure

2.1. Preparation of Magnesium Oxide Nano Flakes

Q-switched Nd: YAG laser (Huafei Tongda Technology-DIAMOND-288 pattern EPLS) was used in our experimentation to ablate of magnesium in liquid. The laser specification was (a wavelength of 1064 nm, frequency equal to 1 Hz, pulse width of 10 ns, and 1000 mJ laser energy). The high-purity magnesium ribbon illustrated in Fig. 2 a, (99.95%, BDH chemical Ltd pool, England) was used to produce magnesium oxide NFs by pulsed laser ablation in water (PLA) method. The magnesium ribbon was cut into strips and lined up together for use in the ablation process as seen in Fig. 2 b. Magnesium strips were cleaned with methanol and then washed using ultrapure deionized water. After careful cleaning, the Mg strip was used as a target for the PLA experiments

and essentially immersed in 5ml of distilled water (DW) in the bottom of a glass container at room temperature. A schematic image summarizes the PLA technique in Fig. 2 d. Because of the high laser energy focused on the target, the irradiated area suffered from the fast removal of material confined to the laser spot. The liquid in the container was moved using a magnetic stirrer to avoid shielding effects from the plume due to the plasma in the static liquid and to guarantee homogeneous dispersion of the ejected nanoparticles. The plasma formed by PLA of the target placed in liquid is considered a source of additional force to produce smaller nanoparticles. The interaction of the ablated species with the laser beam may become a source for secondary ablation force, splitting the colloidal species, or even the formation of complex chemical compounds. The DW color turned to milky white resulting from the ablation process to the magnesium target, as seen in Fig. 2 c.

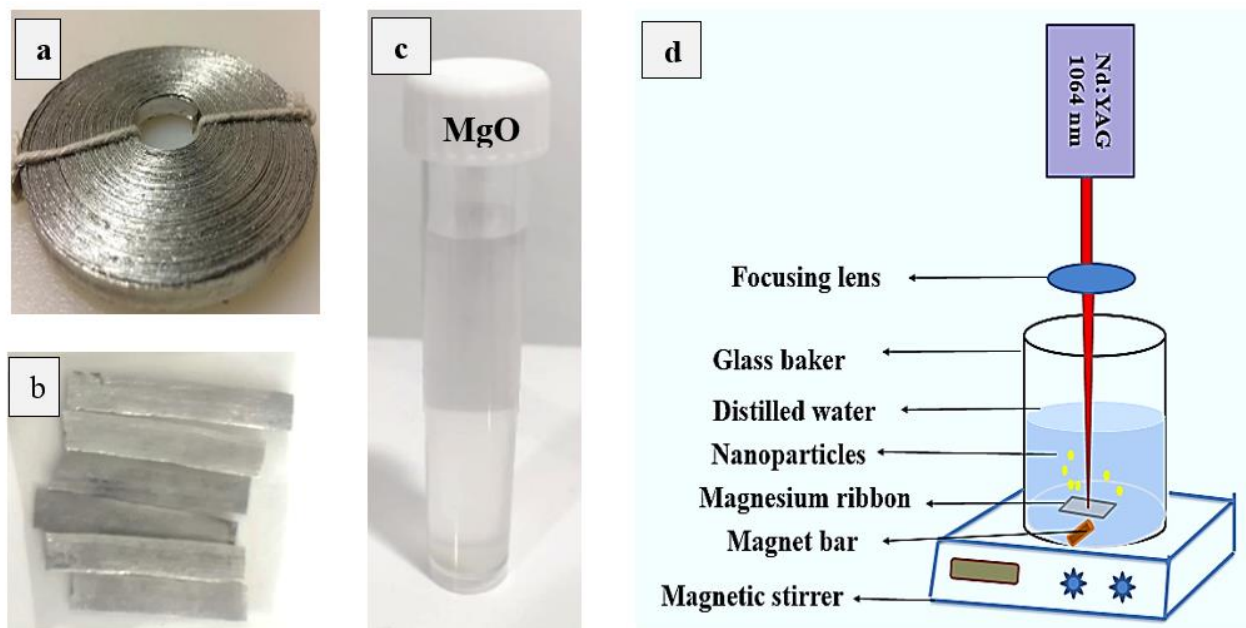


Figure2: (a) magnesium ribbon (b) magnesium ribbon after cutting (c) the white color of the sample after ablation (d) schematic for the manufacture of NFs by PLA of a target in DW.

2.2. Characterization of MgO NFs

MgO NFs were described by X-ray diffraction (XRD) (AERIS PAN alytical, japan). XRD analyses were performed at 40 kV and 30 mA with CuK at 1.5406 Å wavelengths. The samples were examined in two-step increments of 0.05°/s between 20° and 80°. The bond vibrations of NFs were measured using Shimadzu 8000 Series Fourier transform infrared (FTIR) spectroscopy in the 400-4000 cm⁻¹ range. The optical absorbance of colloidal nanoparticles (200-800) was estimated using a UV VIS spectrophotometer with a twin beam (Shimadzu UV-1800). The Axia ChemiSEM scanning electron microscope was used to analyze the morphology of the nanostructures. The chemical composition of the material is investigated using X-ray energy dispersions (Axia Chemi). To validate zeta potential and particle diameter, dynamic light scattering (DLS) (Malvern Zetasizer ZS, Malvern, UK) is utilized.

2.3. Antibacterial Activity

Antibacterial activity of the MgO NFs was carried out by agar well diffusion method against two pathogenic bacterial strains namely *S. aureus* (gram-positive) and *E. coli* (gram-negative). Mueller Hinton Agar was used as a nutrient broth. The bacteria had been previously isolated and characterized from Shariati Hospital-Tehran-Iran. The desired bacteria (*S. aureus* or *E. coli*) were dispersed on the Petri dish's surface. Wells at 6 mm diameter were punched out of each agar plate using a sterilized well cutter. After using an electrical spinner to combine the MgO NFs, Different concentrations of the tested MgO NFs (25, 50, 100, 150, and 200) µg/ml were supplementary in a separate hole in the well. Finally, after incubation at 37 °C for 24 h, the inhibition zones were observed around each well and measured (to the nearest mm) to determine the antibacterial activity of MgO NFs.

2.4. Statistical Analysis

The acquired data were statically analyzed using an unpaired t-test with GraphPad Prism 6. The values were presented as the mean \pm SD (*p < 0.05; **p < 0.01; ***p < 0.001); n = 3).

3. Results and Discussions

The crystal phases and crystallinity of prepared MgO NFs were investigated by XRD analysis. Two samples of the prepared MgO NFs (10 ml) were dropped on a glass slide by vaporizing the NFs suspension (thickness equal 400 nm approximatly). We took this step to obtain a suitable thickness for X-ray diffraction examination. A typical XRD pattern for the manufactured MgO NFs presented in Fig. 3. Miller indices indicated recorded on top of each diffraction peak. The peaks obtained at 38.86°, 59.46°, 62.83°, and 73.87° attributed to the diffraction from (111), (110), (220), and (311), respectively. These peaks are attributed to the formation of MgO NFs. This demonstrates that the polycrystalline cubic structure of MgO NFs formed. The broadening peaks appeared in the XRD figure. This is related to the XRD peaks that broaden as the crystallite size reduces from bulk to nanoscale dimensions [44]. The peak resultant is an agreement to the cards (JCPDS card, No. 45-0946) and (JCPDS No. 4-829).

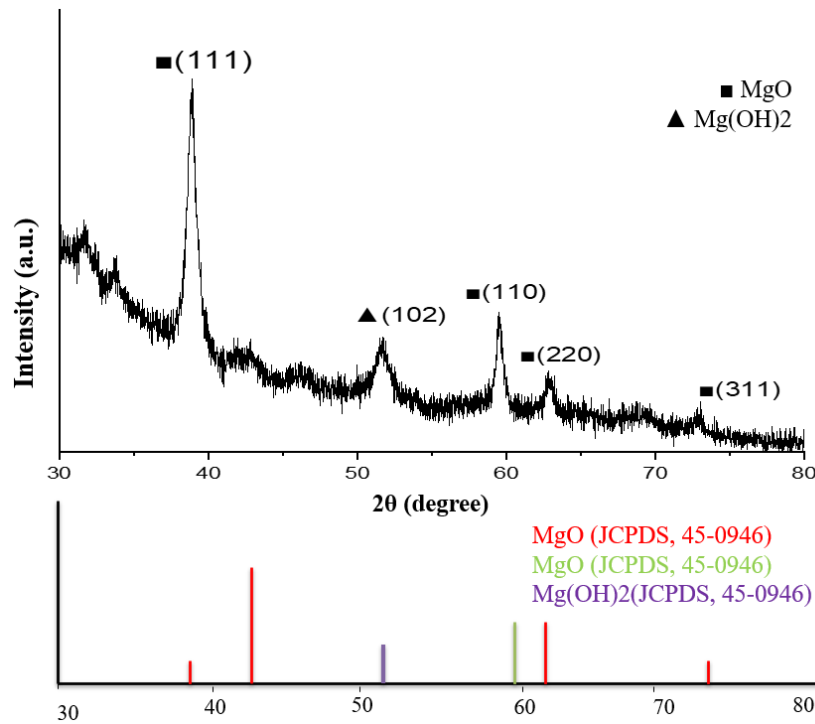


Figure 3: Showing the XRD of MgO NFs deposited on a glass substrate.

Table 1, explains the analyzed sample's orientation, crystalline size, and d-spacing in detail. The peak observed at 2θ equal to 51.56° is associated with the (102) plane of $\text{Mg}(\text{OH})_2$ card no. JCPDS No. 45-0946 [45]. The reason for the presence of magnesium hydroxide related to that $\text{Mg}(\text{OH})_2$ is easily formed when MgO is hydrated, covering the MgO surface[46]. The employee determines the average crystalline size of the Scherrer formula [45, 47] as shown in equation (4):

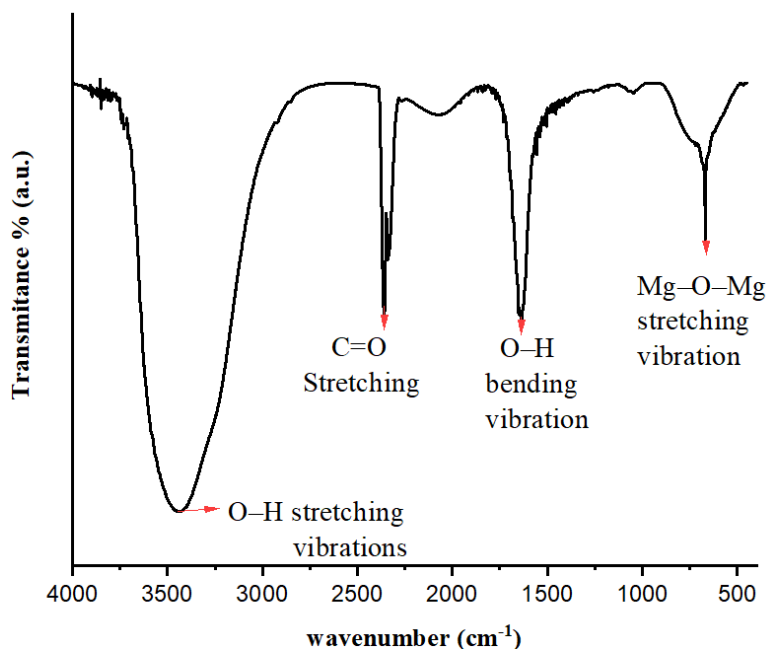
$$D = \frac{0.9\lambda}{\beta \cos\theta} \quad (4)$$

Where D is the average crystalline size, 0.9 is the shape factor constant, λ is the wavelength used as the source (Cu-K α), β is FWHM, which is the (full width at the half-maximum), θ is the diffraction angle. It can be seen the average size produced for the sample manufactured using the PLA technique was approximately 25 nm.

Table 1: XRD structural parameter of MgO NFs.

POSITION	(HKL)	CRYSTAL. SIZE	D-SPACING	CARD NO.	REF.
38.86	(111)	27.9 nm	2.31536 Å	(JCPDS card, No. 45-0946)	[48]
59.46	(110)	41.0 nm	1.5531 Å	(JCPDS No. 4-829)	[49]
62.83	(220)	17.4 nm	1.47775 Å	(JCPDS card, No. 45-0946)	[50]
73.87	(311)	16.1 nm	1.29698 Å	(JCPDS card, No. 45-0946)	[51]

Using FTIR spectroscopy, Balamurugan et al. [52] studied the MgO NFs and showed the vibrational mode of Mg–O–Mg located between $(487\text{--}677)\text{ cm}^{-1}$ wavenumber region. On the other hand, the previous studies show the MgO stretching vibration mode $\sim 600\text{--}850\text{ cm}^{-1}$ [45, 53]. In this study, the peak between 640 cm^{-1} and 715 cm^{-1} is correlated to Mg–O–Mg bonds that confirm the creation of MgO NFs and are indicated with the mentioned references. Water molecules' O–H stretching vibrations are related to the wide vibration band at $3440\text{--}3450\text{ cm}^{-1}$, whereas those at $1630\text{--}1640\text{ cm}^{-1}$ are connected with their bending mode [42]. Furthermore, a faint band related to gas-phase CO_2 adsorption is evident at roughly $2375\text{--}2385\text{ cm}^{-1}$ due to carbon dioxide from ambient or lab settings. FTIR spectrum of MgO NFs prepared using laser ablation method displayed in Fig. 4.

**Figure 4:** FTIR spectra of MgO NFs suspension prepared using laser ablation in liquid method.

A visible UV spectrophotometer was used to record UV VIS spectra of the synthesized MgO NFs suspension in the absorbance mode represented in Fig 5a. The wavelength ranges between $200\text{--}800\text{ nm}$ to determine the absorbance of MgO NFs. MgO NFs absorption region has a wide range beginning from 250 nm to 800 nm . The reality that the peak doesn't appear sharp suggests that this approach produces varying-sized nanoparticles [54]. Tauc's relation [55] can be used to specify the relationship between the photon energy ($h\nu$) and the coefficient (α), which was:

$$(\alpha h\nu) = A (h\nu - E_g)^n \quad (5)$$

Where A is a constant relay to the type of the material, E_g is band gap energy, and 'n' is a constant, which is an exponent whose values depend on the electronic transition and range from $1/2$ to $3/2$, 2 to 3. For the current

investigation, the optical band gap was obtained using $n = 2$ (for the permitted direct transition band gap) where $n = 1/m$ [56]. The absorption coefficient (α) of the magnesium oxide is valued using the mathematical formula [57]:

$$\alpha = 2.303 \frac{A}{d} \quad (6)$$

Where: d is the path length and A absorbance related to the suspended MgO NFs. The band gap of MgO NFs was calculated by plotting the $(\alpha h\nu)^2$ versus $h\nu$ (eV) as revealed in Fig. 5 b. For MgO NFs, a plot between $(\alpha h\nu)^2$ and photon energy ($h\nu$) is drawn, considering the direct band transition in MgO NFs. The tangent's intercept shows the direct band gap of MgO NFs to the plot on the X-axis. The optical energy band gap value for the MgO NFs samples (5.8 eV), according to Fig. 5 b matched with the previous reference [58]. In the current work, MgO has an optical band gap greater than the bulk value attributed to the quantum confinement effect. The intergranular areas' chemical vacancies or flaws, which create new energy levels to raise the band gap energy, are responsible for this impact [59, 60].

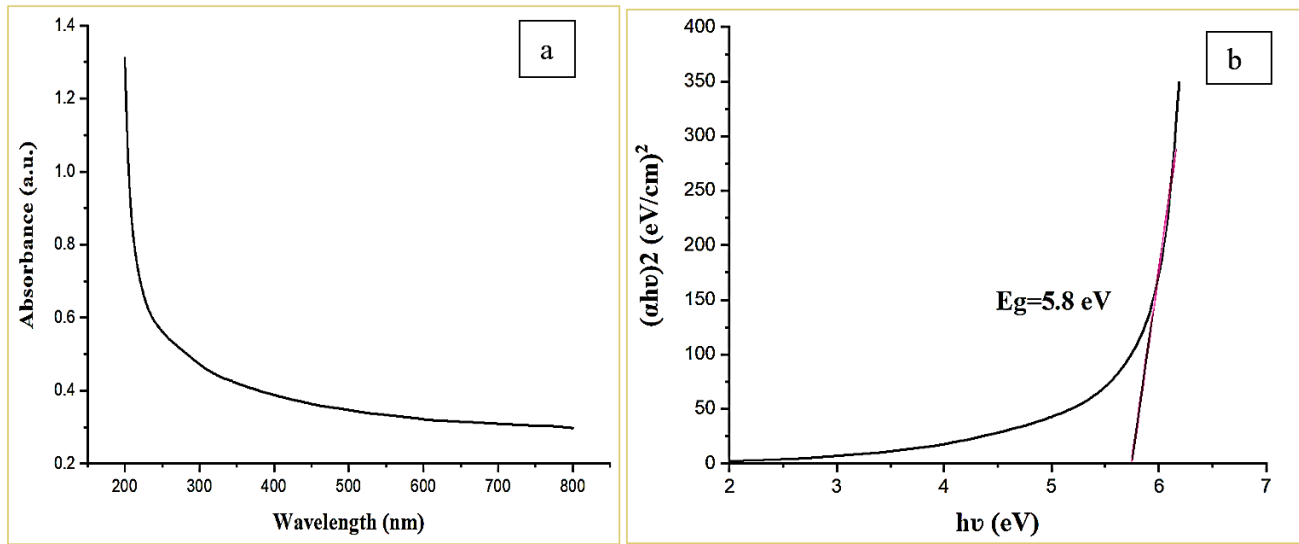


Figure 5: Illustrate (a) UV visible spectra of MgO NFs, (b) estimated band gap.

FESEM images of the MgO NFs suspension deposited on the glass slab are shown in Fig. 6 a. Morphological analysis shows irregular distribution clusters of flake-like nanostructures. These structures sometimes appear branched in nature. These nano flake structures have a typical diameter of 100-400 nm and a very small thickness of the walls around 24 nm. It demonstrates clearly that the vertically aligned MgO NFs grow uniformly on a glass substrate. Most NFs intersect to form a network structure providing a large surface area. It is well documented in the literature that laser ablation in liquid normally produces nanoparticles, with most having sphere-like structures. In the present work the production obtained was nano flakes. Laser ablation method with very high laser energy may exfoliate the surface of magnesium target. This is mostly because the target has a low melting point (650 °C), boiling point (1107 °C), and hardness point [61] hence low ablation threshold. Another proposed reason related to the aging time may reconstructed the structure from spherical to nanorod or nanocubes or nano flakes. The disappearance of the surface plasmon resonance peak support the last reason [62].

The EDX analysis shown in Fig. 6 b indicated the existence of Magnesium (Mg), and Oxygen (O) elements in the sample at bending energy 0.5–1.5 keV without any other impurities. This proves the high purity of the manufactured MgO NFs using the laser ablation technique. In the MgO NFs sample, the elemental compositions of O, and Mg were 59.6 %, and 40.4%, respectively.

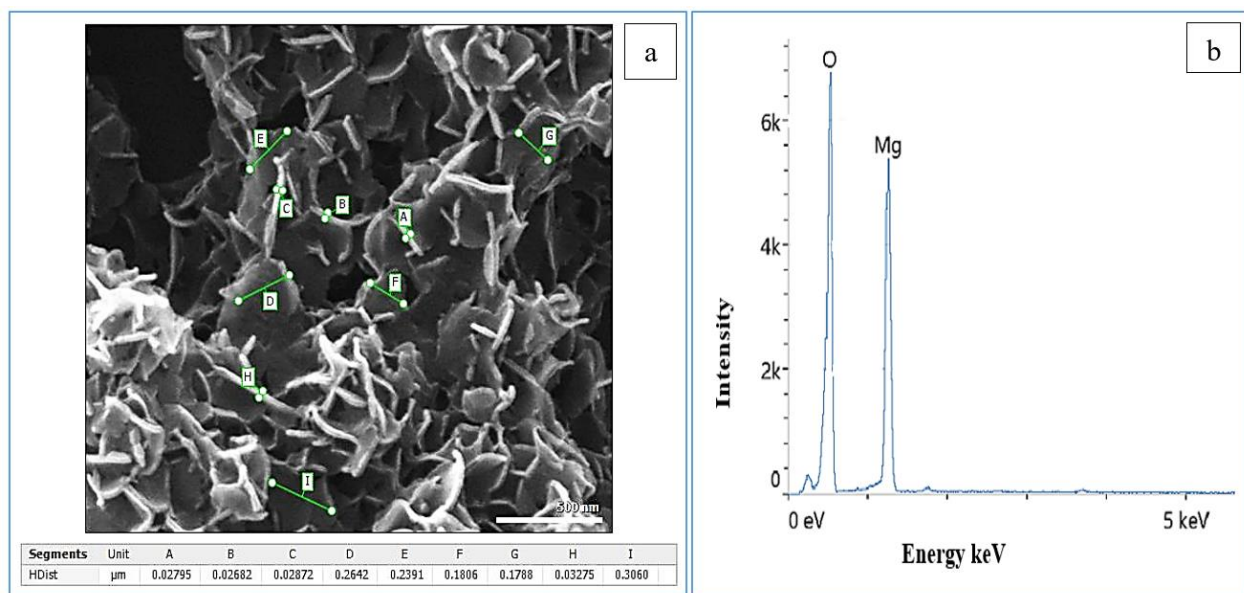


Figure 6: (a) shows the FESEM image of MgO NFs, (b) EDX analysis of composition MgO NFs.

Diffraction light scattering (DLS) was used to identify the hydrodynamic residue and distribution of sizes of MgO NFs in colloidal dispersion illustrated in Fig. 7 a. According to the size distribution graph, the average size of the MgO NFs in the dispersion of colloids was 30.59 nm. As demonstrated, the average size of MgO NFs changed depending on the analysis. The particle size determined by DLS was greater than that determined by FESEM and XRD. The observed result may be explained by the variations in the FESEM and DLS analysis circumstances. DLS measurements are carried out in an aqueous environment to account for the difference in particle diameter determination, and FESEM analysis is carried out on dry particles. The hydrodynamic diameter, which represents the size of the particles when they are hydrated, is provided by DLS. The poly-dispersity index of the NFs is provided by the DLS analysis. The DLS analysis provides the poly-dispersity index of the NFs. The range of the PDI scale is 0 to 1 [27]. The suspension homogeneity is indicated by a PDI score of less than (0.4). However, a PDI value of more than one means the suspension is extremely diverse [63]. In this study, the PDI provided was 0.07. This suggests the synthesized NFs' particle size distribution was more homogeneous and narrower. This implies that a sizable fraction of the MgO NFs had comparable sizes, which helped to create a well-defined size distribution. Improved control over the synthesis process, which produces NFs with uniform sizes, is frequently implied by a lower PDI value. The present PDI value is encouraging in general as it suggests that the synthesis procedure produced MgO NFs with comparatively consistent particle sizes. This could be useful for integrating the particles into different applications where a uniform particle size is required. The zeta potential represents the movement of MgO NFs in a dispersion of colloidal particles under the effect of an electric field. In the present investigation, Fig. 7 b shows the zeta value (- 36.1 mV) for PLAL MgO NFs, suggesting good stability. The highly stable dispersions present Zeta values of more than ± 30 mV. This evidence suggests increased electrostatic repulsion between NFs, assisting in stabilizing and preventing their aggregation. Because of the significant negative charge, there is more electrostatic repulsion between the particles, which lessens the potential for the particles to aggregate and ensures their stability.

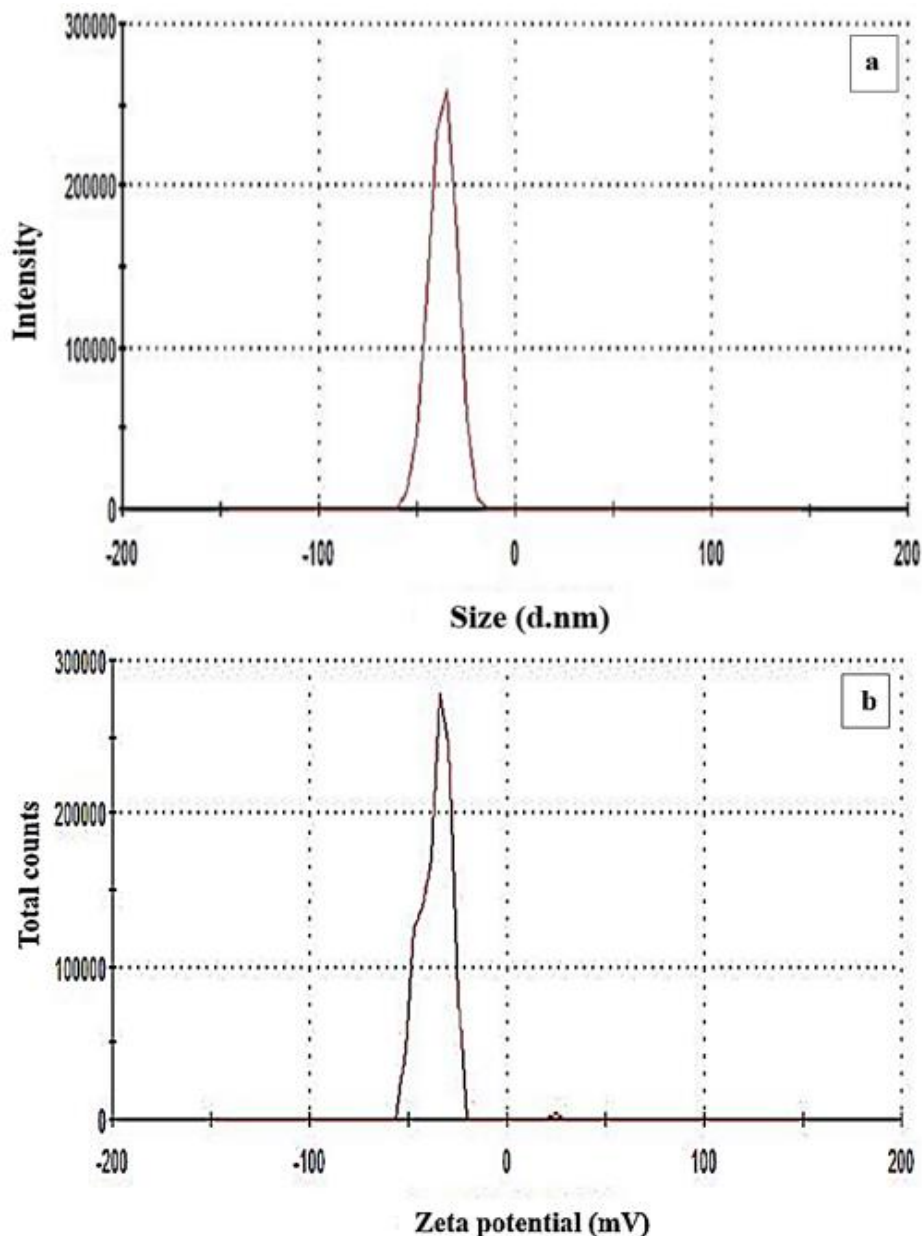


Figure 7: DLS analysis of MgO NFs (a) particle size, (b) zeta potential.

4. Antibacterial Test

Multi-drug resistant microorganisms (MRM) are a significant concern in both agriculture and healthcare. These MRM strains have sophisticated mechanisms that allow them to withstand the effects of antibiotics, making it more difficult to treat and manage their infections. To improve their antimicrobial action, scientists are actively researching new active chemicals like nanoparticles, which have distinct qualities such as their vast surface area, compact size, and capacity to penetrate microbial cells [27, 64]. Magnesium oxide nanoparticles in this work show impressive antibacterial efficacy against Gram-positive and Gram-negative bacteria. It was discovered that the degree of bacterial growth inhibition seen in this investigation varied and depended on concentration. This phenomenon was consistent with several previously published investigations [26, 65]. Fig. 8 shows the antibacterial activity of MgO NFs against *E. coli* and *S. aureus* isolates in different concentration. Different concentrations of MgO NFs (25, 50, 100, 150, and 200) $\mu\text{g/ml}$ were applied to verify the antibacterial activity. The first column represents pure distilled water, and the final one refers to silver nitrate (AgNO_3) as a control agent. This step was done to compare with the antibacterial activity of MgO NFs. Using the concentrations (150 and 200) $\mu\text{g/ml}$ exceeded the effect of AgNO_3 . As shown, the highest antibacterial activity was observed at a high MgO NFs concentration (200 $\mu\text{g/ml}$) with inhibition zones 15 mm $\pm 0.5\text{mm}$ for *E. coli* and 16 mm $\pm 0.5\text{ mm}$ for

S. aureus. The inhibition zone decreased remarkably to $5 \text{ mm} \pm 0.5 \text{ mm}$, and $4 \text{ mm} \pm 0.5 \text{ mm}$ at a concentration ($25 \text{ } \mu\text{g/ml}$) for *E. coli* and *S. aureus*, respectively. The minimum inhibitory concentration (MIC) value denotes the smallest amount of the active ingredient required to slow or prevent microbial growth. Determination of (MIC) is a crucial step in investigating the efficacy of active substances and comprehending their ability to combat various pathogenic microorganisms. The choice of antibiotics, concentration optimization, reducing the emergence of antibiotic-resistant microbes, contrasting the antibacterial activities of different compounds, identifying new tactics for battling infectious diseases, aiding in drug formulation and clinical choice, and helping to develop efficient treatment protocols all rely heavily on MIC data [27, 66]. In this study, the MIC for MgO NFs prepared using laser ablation in liquid technique was $25 \text{ } \mu\text{g/ml}$ for both pathogens studied. The minimum inhibitory concentration was $25 \text{ } \mu\text{g/ml}$ for both pathogens studied. The structure of microbial cell walls is a major factor in explaining MgO NFs' antibacterial action. Compared to Gram-negative bacteria, gram-positive bacteria have a thicker peptidoglycan layer that serves as a barrier preventing MgO NFs from penetrating [67, 68]. This explains why MgO NFs have greater antibacterial action against gram-negative bacteria than they do against gram-positive bacteria.

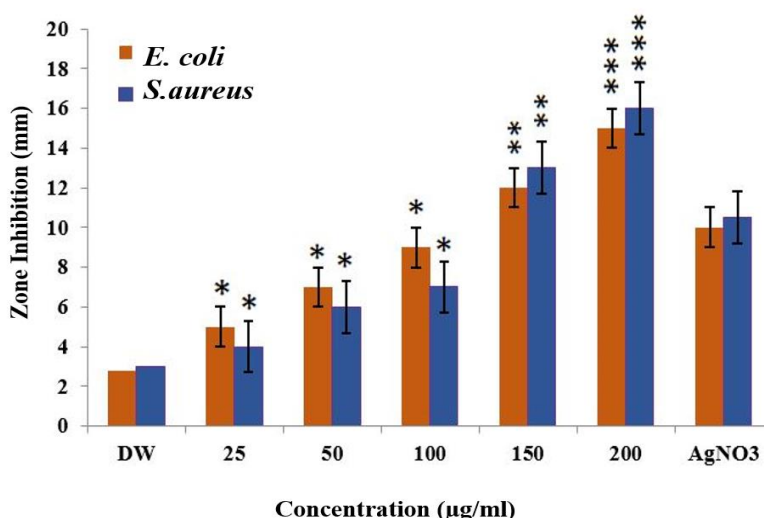


Figure 8: Antibacterial activity of MgO NFs against *E. coli* and *S. aureus*. (DW: distilled water, AgNO₃: control agent). Data are presented as (mean \pm SD (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$); $n = 3$).

5. Conclusion

Pulse laser ablation in liquid could be considered the best and easiest way to create MgO NFs. The results of the characterization technique (XRD, UV visible, FESEM, EDX, DLS, and FTIR) confirm the creation of MgO NFs. These nanostructures were poly crystalline, flake-like in shape, had different diameters reaching 400 nm with an average thickness of approximately 24 nm, and the band gap obtained was wide reach to 5.8 eV. MgO NFs have significant negative charge and high stability. MgO NFs showed remarked antibacterial action versus the two bacterial strains (*S. aureus* and *E. coli*) with low MIC values ($25 \text{ } \mu\text{g/mL}$).

Conflict of Interest

The authors declare that they have no conflict of interest.

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