

Influence of laser ablation parameters on the synthesis, characterization, and antibacterial activity of iron oxide nanoparticles

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ABSTRACT

Pulsed laser ablation in liquid (PLAL) is a simple and rapid technique that enables the synthesis of nanoparticles (NPs) with high purity. It is a top-down physical approach based on the fragmentation of a bulk metal precursor. In this work, iron oxide nanoparticles (Fe_3O_4 NPs) were synthesized using PLAL with an Nd laser at a pulse energy of 500 mJ. Two ablation wavelengths, 355 and 532 nm, and different pulse numbers, 500, 600, 700, 800, and 900 pulses, were used for both wavelengths to evaluate their effects on the optical properties of the synthesized NPs. Structural and morphological characterization was performed for the NPs synthesized using the highest number of pulses, 900 pulses, using several analytical techniques. The antibacterial activity of all synthesized NPs was then evaluated against four bacterial species isolated from the oral cavity: *Streptococcus mutans*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*. The results showed that the synthesized NPs were spherical and exhibited a cubic polycrystalline phase, with average crystallite sizes of 17.47 and 20.39 nm for the samples synthesized at 355 and 532 nm, respectively. The lower ablation wavelength resulted in smaller average crystallite size, higher absorbance, and greater antibacterial activity compared with the longer wavelength. These findings indicate that Fe_3O_4 NPs synthesized by PLAL have antibacterial potential and may be considered for further investigation as supportive antimicrobial agents and for other biomedical applications.

Keywords: Antibacterial activity, Antibiotic alternative, Fe_3O_4 NPs, Laser ablation, Nano-colloidal

1 INTRODUCTION

Pulsed laser ablation in liquid (PLAL) is a physical approach that is considered a rapid, economical, and sustainable method for synthesizing nanoparticles (NPs). This technique can contribute to the development of nanomaterials for addressing several biomedical and health-related challenges [1]. In recent decades, the increasing virulence of pathogenic bacteria and their resistance to conventional antibiotics have become major threats to human health. Therefore, there is an urgent need to develop alternative or supportive antimicrobial

strategies, including the use of NPs [2].

Nanoparticles possess a high surface-area-to-volume ratio [3–6], making them suitable for various biomedical applications. They can interact effectively with biomolecules and biological systems [7, 8]. NPs have received considerable attention because of their unique physicochemical properties, including chemical stability, electronic behavior, conductivity, magnetic properties, and potential biomedical applicability [9–11]. Metal NP colloids are of significant interest to researchers in different scientific disciplines [12]. One of their important optical features is surface plasmon resonance

(SPR) [6]. When the surface of metal NPs is exposed to light of suitable fluence and wavelength, free electrons can collectively oscillate, producing wavelength-dependent absorption and scattering. These plasmonic effects can enhance the local electromagnetic field around the particles and produce measurable optical changes [13, 14].

Among metal-based nanomaterials, iron and iron oxide NPs have attracted considerable attention in modern technology because of their optical, mechanical, catalytic, electrical, magnetic, and general physical properties [11]. Recently, laser ablation has been adopted as a flexible, economical, and versatile technique for synthesizing nanoparticles, including iron oxide NPs [12–14]. One of the most important biomedical applications of iron oxide NPs is their antibacterial activity [2]. Their antibacterial mechanisms may include interaction with negatively charged bacterial cell-wall components, destabilization of the cell membrane, changes in membrane permeability, and generation of oxidative stress [15].

Laser ablation can produce highly pure NPs without the need for toxic chemical reducing or stabilizing agents [16]. This physical method is based on the top-down fragmentation of a bulk metal precursor into nanosized particles [9]. This provides an important advantage for biological applications, where NPs with clean surfaces and minimal contamination by residual reactants are required. In addition, PLAL can reduce preparation and processing costs [17, 18].

Several laser parameters affect the response of the target material during ablation, including laser wavelength, pulse energy, pulse number, repetition rate, and irradiation time. Continuous laser irradiation can induce a sequence of thermal and mechanical processes, including melting, boiling, evaporation, plasma formation, phase explosion, stress generation, and deformation of the target material. The liquid ablation medium also affects nanoparticle formation and the optical properties of the colloidal solution. The position and intensity of the optical absorption peak can be modified by changing the ablation wavelength, number of pulses, repetition rate, pulse energy, and other experimental conditions [19].

Metal oxide NPs possess distinctive physical properties, catalytic activity, and antibacterial effects. These properties are strongly influenced by particle size, shape, spatial arrangement, and synthesis conditions [14]. Therefore, this work aims to synthesize iron oxide nanoparticles using laser ablation as a rapid, low-cost, and environmentally friendly method. It also investigates the effect of ablation parameters on their physical properties

and antibacterial activity to evaluate their potential as supportive antimicrobial agents.

2 MATERIALS AND METHODS

Nanocolloidal solutions containing Fe_3O_4 NPs were prepared using a pure iron sheet with dimensions of $10 \times 10 \times 2$ mm. The iron sheet was immersed in a beaker containing 5 mL of deionized water and positioned 5 cm from the Q-switched Nd laser source. The laser was operated at a repetition rate of 6 Hz, pulse energy of 500 mJ, and wavelengths of 355 and 532 nm. Different pulse numbers, namely 500, 600, 700, 800, and 900 pulses, were used for each wavelength. Accordingly, ten nanocolloidal samples containing Fe_3O_4 NPs were obtained.

The NPs synthesized at 900 pulses for each wavelength were subjected to structural and morphological characterization using X-ray diffraction (XRD), atomic force microscopy (AFM), and field-emission scanning electron microscopy (FESEM). For these analyses, the colloidal solutions were deposited on glass slides using the drop-casting method, in which small drops of the nanoparticle suspension were placed on the substrate and allowed to dry, forming thin films [20].

All ten prepared samples were analyzed optically using UV-visible spectrophotometry. Their antibacterial activity was evaluated against four bacterial species isolated from the oral cavity. Two Gram-positive bacteria, *Streptococcus mutans* (*S. mutans*) and *Staphylococcus aureus* (*S. aureus*), and two Gram-negative bacteria, *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Escherichia coli* (*E. coli*), were tested. The antibacterial assay was performed using the agar well-diffusion method. Briefly, bacterial suspensions were spread on agar plates, and 10 μ L of each prepared nanocolloidal solution was added to wells formed in the agar. The plates were incubated at 37 °C for 24 h. Antibacterial activity was evaluated by the appearance and diameter of inhibition zones around the wells [11, 13].

The antibacterial activity of the synthesized nanoparticles was compared with that of fifteen antibiotics: amikacin (AK), amoxicillin (AM), ampicillin (AX), azithromycin (AZM), cefotaxime (CTX), cefixime (CFM), ciprofloxacin (CIP), erythromycin (E), gentamicin (CN), imipenem (IPE), levofloxacin (LEV), meropenem (MEM), nitrofurantoin (F), streptomycin (S), and trimethoprim/sulfamethoxazole, also known as cotrimoxazole (TS).

3 RESULTS AND DISCUSSION

The structural characterization results of the prepared samples confirmed the formation of Fe_3O_4 nanoparticles at the nanoscale. The XRD patterns shown in Figure 1 indicate the formation of polycrystalline Fe_3O_4 NPs with a cubic phase. For the NPs synthesized at an ablation wavelength of 355 nm and 900 pulses, diffraction peaks were observed at 2θ values of 32.55° , 35.75° , 44.55° , 54.55° , 64.35° , 57.45° , and 75.05° . For the NPs synthesized at 532 nm and 900 pulses, the corresponding peaks appeared at 32.45° , 36.65° , 43.95° , 55.35° , 65.35° , 56.00° , and 75.15° . These peaks were assigned to the Miller indices (220), (311), (400), (422), (511), (440), and (533), respectively, and showed good agreement with JCPDS card No. 11-0614.

The shorter ablation wavelength of 355 nm produced NPs with a smaller average crystallite size of 19.78 nm compared with those synthesized at 532 nm, which showed an average crystallite size of 22.71 nm. This reduction in crystallite size may be attributed to the higher laser fluence associated with the shorter wavelength, which promotes the formation of new nucleation centers and leads to smaller crystallite sizes. This result is consistent with previous findings [21].

The average crystallite size, D , was calculated using the Debye-Scherrer equation [14], based on the X-ray wavelength, λ , the diffraction angle, θ , and the full width at half maximum, β , of the diffraction peaks:

$$D = \frac{0.9\lambda}{\beta \cos \theta} \quad (1)$$

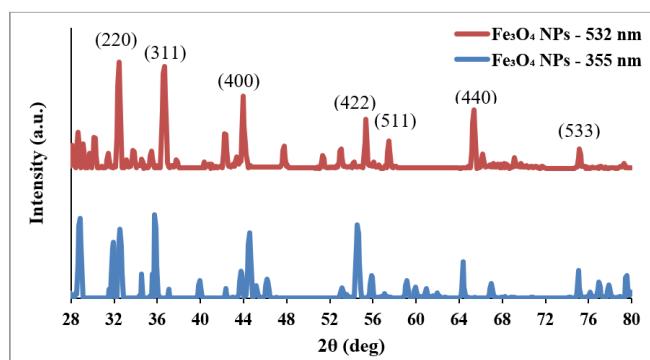


Fig. 1 XRD patterns of Fe_3O_4 NPs synthesis at the wavelengths (355&532 nm) and (900 pulses)

The XRD patterns showed several additional peaks, which may be attributed to different factors. Some of

these peaks may be related to the material itself, such as the formation of additional crystalline phases, indicating partial transformation of the material into another oxide phase. Other peaks may arise from instrumental factors, measurement conditions, or peak overlap.

Laser-induced oxidation is an important process during the ablation of metals. This oxidation can occur because of the high local temperatures generated by laser irradiation and the rapid interaction of the ablated species with oxygen-containing species in the surrounding medium. The dissipated heat is rapidly transferred to the surrounding environment, causing thermal diffusion into regions adjacent to the laser-irradiated area. The ablated material then melts, evaporates, and expands rapidly into the plasma plume, where it can react with oxygen-containing species. Therefore, this thermal and plasma-assisted process can contribute to the oxidation of the resulting NPs [22]. In other studies, the formation of metal oxide nanoparticles has been attributed to the interaction between metal atoms and oxygen atoms or free radicals produced during dissociation of the liquid medium. The type of oxide formed depends on temperature, oxygen availability, and the local reaction environment [10].

FESEM images of the synthesized Fe_3O_4 NPs were used to examine nanoparticle morphology. Figures 2 and 3 show the NPs synthesized at 900 pulses using ablation wavelengths of 355 and 532 nm, respectively. The synthesized particles appeared mostly spherical. The average particle size generally ranged from 44 to 59 nm for the sample synthesized at 355 nm and from 52 to 64 nm for the sample synthesized at 532 nm. Although some agglomeration was observed in both samples, the particle dimensions remained within the nanoscale range. The average particle size was smaller for the sample prepared using the shorter ablation wavelength of 355 nm, which is consistent with previous findings [23].

Figures 4 and 5 present the AFM analysis of the NPs synthesized using 900 pulses. The samples prepared using the shorter wavelength of 355 nm appeared more uniform and denser than those prepared at 532 nm. Surface topography analysis showed that increasing laser fluence reduced the grain size. The average grain size of the NPs synthesized at 355 nm was 55.27 nm, whereas that of the NPs synthesized at 532 nm was 86.57 nm. In addition, the cumulative grain-size distribution of the nanoparticles synthesized at the shorter wavelength was more regular, in agreement with previous findings [23].

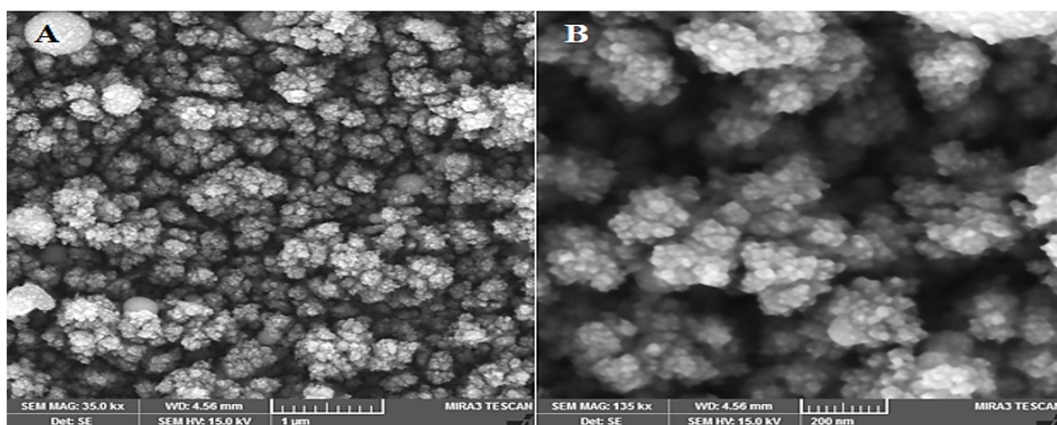


Fig. 2 FESEM images of Fe₃O₄NPs at the wavelength 355 nm with the two zoom at: (A) 1 μm, (B) 200 nm

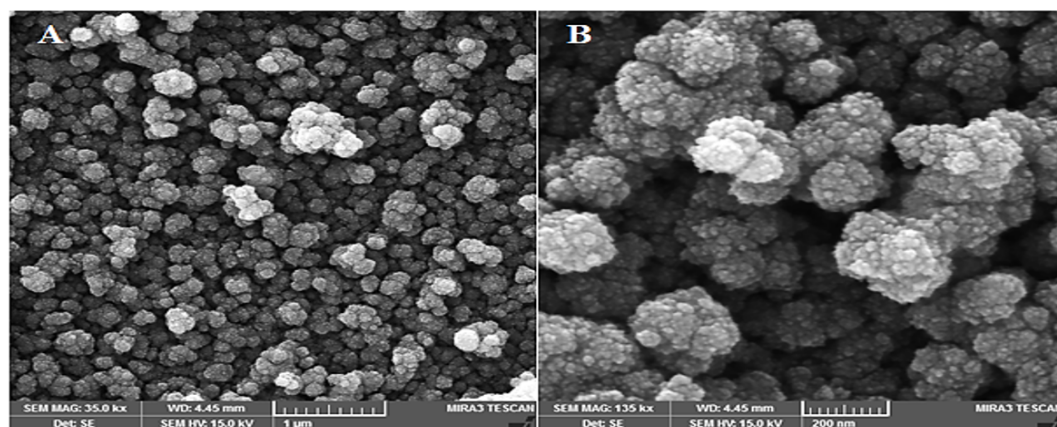


Fig. 3 FESEM images of Fe₃O₄NPs at the wavelength 532 nm with the two zoom at: (A) 1 μm, (B) 20 nm

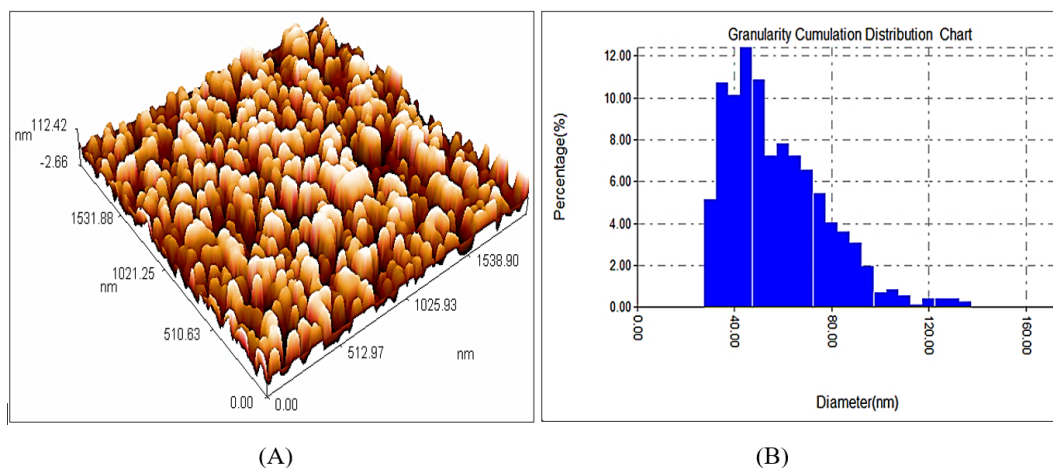


Fig. 4 AFM analysis of Fe₃O₄NPs at 355 nm : (A) 3D image, (B) Distribution of the granularity cumulative

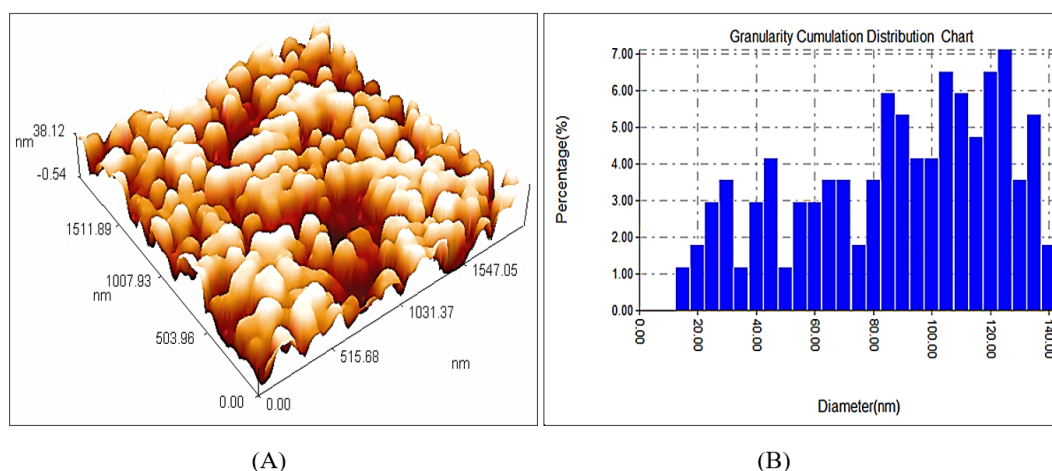


Fig. 5 AFM analysis of $\text{Fe}_3\text{O}_4\text{NPs}$ at 532 nm : (A) 3D image, (B) Distribution of the granularity cumulative

The UV-Vis spectroscopy results are represented by the absorption spectra and optical band gaps of the synthesized NPs. The absorption spectra shown in Figure 6 for colloidal solutions containing Fe_3O_4 NPs prepared at 355 nm exhibited smooth and regular curves, indicating a relatively uniform size distribution and morphology, as the optical properties of nanomaterials generally depend on their structural characteristics.

It is also clear that increasing the number of laser pulses increased the absorbance. This increase can be attributed to the higher concentration of synthesized NPs in the colloidal solution. The absorption intensity increased gradually as the pulse number increased from 500 to 800 pulses. However, at 900 pulses, the absorbance decreased, which may be attributed to saturation of the nanocolloidal solution, leading to agglomeration and physical bonding between NPs. This increases particle size and consequently reduces absorption efficiency. In general, the increase in NP absorbance with increasing laser irradiation is consistent with previous findings [24].

Figure 7 shows the absorption spectra of $\text{Fe}_3\text{O}_4\text{NPs}$ synthesized at 532 nm using different pulse numbers. A variation in absorption intensity was observed with increasing pulse number. As mentioned above, the optical properties of the material depend mainly on its structural properties. The formation of clusters and aggregates on the

nanoparticle surfaces may lead to the accumulation of nanoparticle layers on the ablated target surface, thereby affecting ablation efficiency as the pulse number increases. A comparison between the two ablation wavelengths shows that the 355 nm laser produced higher and broader absorption peaks than the 532 nm laser. In both cases, 800 pulses produced the most favorable absorption behavior. The maximum absorbance peaks appeared in the range of 287-300 nm for NPs synthesized at 355 nm and 301 – 308 nm for those synthesized at 532 nm. The increase in the absorption peak intensity with increasing pulse number may be attributed to the greater production and redistribution of NPs in the colloidal solution, as well as changes in the effective electric layer surrounding the particles [25].

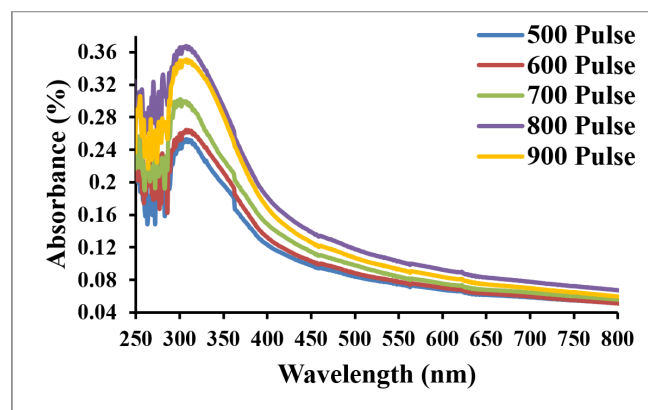


Fig. 6 Absorbance spectra of $\text{Fe}_3\text{O}_4\text{NPs}$ at 355 nm

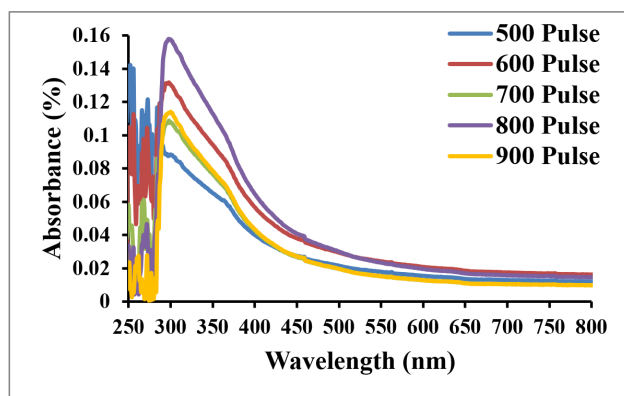


Fig. 7 Absorbance spectra of Fe₃O₄NPs at 532 nm

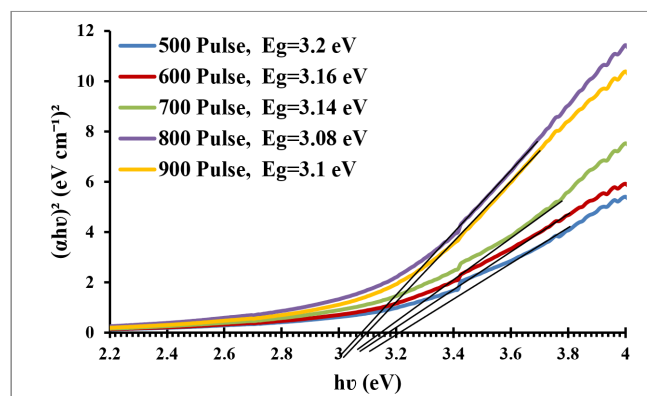


Fig. 8 Energy gap values of Fe₃O₄NPs synthesized at 355 nm

Figures 8 and 9 show the optical energy-gap values of the NPs synthesized at wavelengths of 355 and 532 nm, respectively, for all pulse numbers used in the synthesis. In general, the energy-gap values decreased with increasing pulse number up to a certain limit. This trend is related to the absorbance behavior of the synthesized NPs, where higher absorbance generally corresponds to lower estimated optical energy-gap values.

The energy-gap values ranged from 3.1 to 3.2 eV for the NPs synthesized at 355 nm, which were lower than those obtained for the NPs synthesized at 532 nm, where the values ranged from 3.3 to 3.42 eV. This difference may be attributed to the higher absorbance values observed for the NPs synthesized at 355 nm. The increase in absorbance may result from the higher concentration of synthesized NPs in the colloidal solution, which increases the number of particles and enhances optical absorption. This behavior can contribute to a reduction in the estimated optical energy gap, (E_g). The obtained energy-gap values are comparable with those reported previously [3].

The optical energy-gap values were calculated using the Tauc equation [14]:

$$\alpha h\nu = B (h\nu - E_g)^{\frac{1}{2}} \quad (2)$$

where B is a constant, α is the absorption coefficient, h is Planck's constant, and ν is the photon frequency.

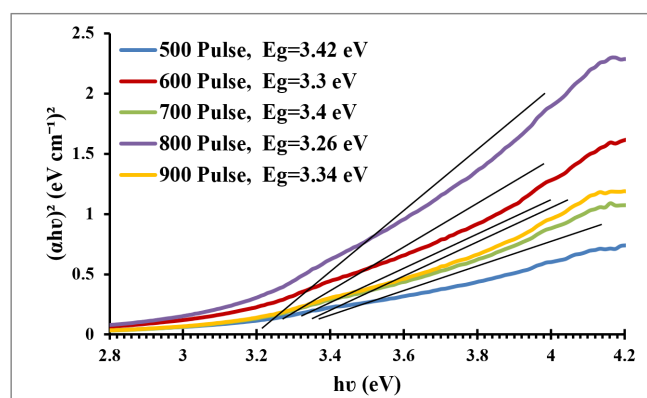


Fig. 9 Energy gap values of Fe₃O₄NPs synthesized at 532 nm

The Fe₃O₄NPs synthesized under these conditions showed antibacterial activity against four bacterial species: *S. mutans*, *S. aureus*, *P. aeruginosa*, and *E. coli*. The antibacterial effectiveness varied depending on the synthesis parameters, particularly the laser wavelength and number of pulses. This activity was evaluated by measuring the inhibition-zone diameters around the wells containing the synthesized NPs, as shown in Figures 10 and 11.

The antibacterial performance depended on the structural and optical properties of the NPs, which were controlled by the ablation parameters, as shown in Figures 12 and 13. The 355 nm wavelength showed greater bacterial inhibition than the 532 nm wavelength, which is consistent with the physical characterization results of the NPs synthesized at 355 nm. Increasing the pulse number generally improved

antibacterial activity, although some variation was observed at higher pulse counts.

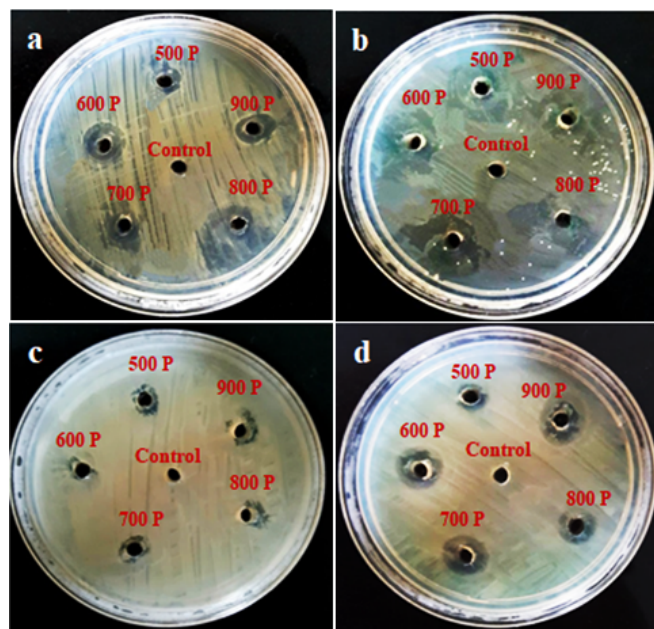


Fig. 10 Antibacterial activity of $\text{Fe}_3\text{O}_4\text{NPs}$ at 355 nm on the bacteria: a) *S. aureus*, b) *S. mutans*, c) *E. coli*, d) *P. aeruginosa*

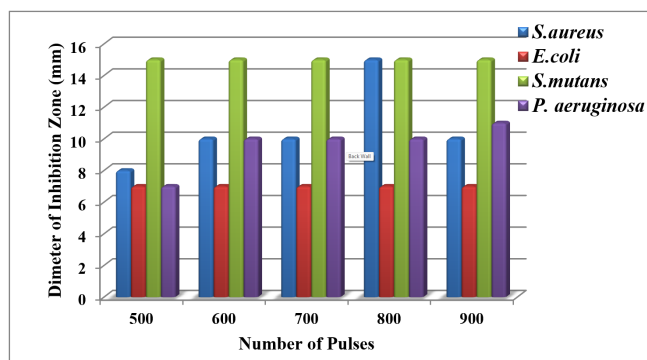


Fig. 12 Histogram of antibacterial effectiveness of $\text{Fe}_3\text{O}_4\text{NPs}$ at 355 nm

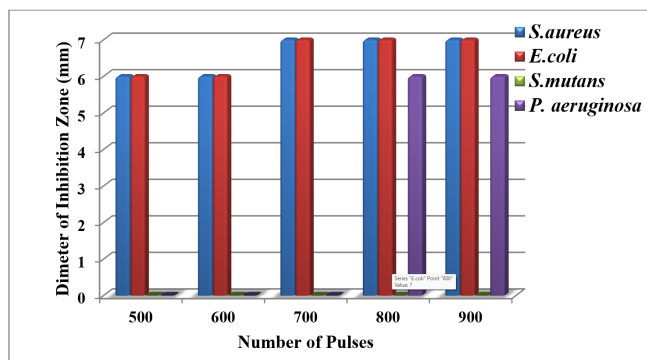


Fig. 13 Histogram of antibacterial effectiveness at of $\text{Fe}_3\text{O}_4\text{NPs}$ at 532 nm

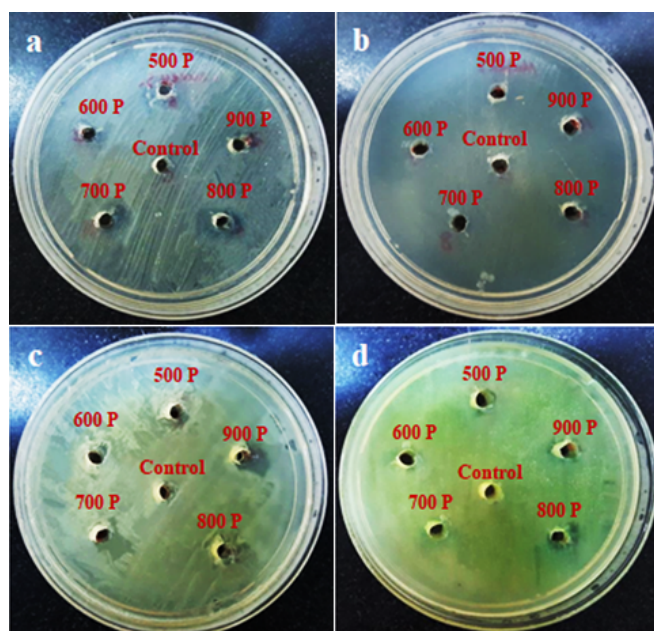


Fig. 11 Antibacterial activity of $\text{Fe}_3\text{O}_4\text{NPs}$ at 532 nm on the bacteria: a) *S. aureus*, b) *S. mutans*, c) *E. coli*, d) *P. aeruginosa*

This trend was consistent with the changes observed in the absorbance spectra. Overall, the combination of 355 nm wavelength and 800 pulses represented the most effective ablation conditions for Fe_3O_4 NP synthesis under the present experimental conditions.

Furthermore, the results showed differences in the sensitivity of the tested bacterial species to the synthesized Fe_3O_4 NPs. *S. aureus* and *E. coli* responded to all prepared samples, whereas *S. mutans* and *P. aeruginosa* responded only to the samples prepared using the 355 nm wavelength. These two species showed resistance to most samples prepared using the 532 nm wavelength, except those synthesized using a high number of pulses.

This variation may reflect differences in bacterial susceptibility, cell-wall structure, and resistance behavior. The antibiotic-sensitivity test shown in

Figure 14 further demonstrated that some bacterial isolates exhibited resistance to conventional antibiotics. This finding supports the main purpose of using synthesized NPs as potential supportive antimicrobial agents, especially because some bacterial

species showed limited or no response to certain antibiotics but responded to synthesized Fe_3O_4 NPs. Similar conclusions have been reported in previous studies [26].

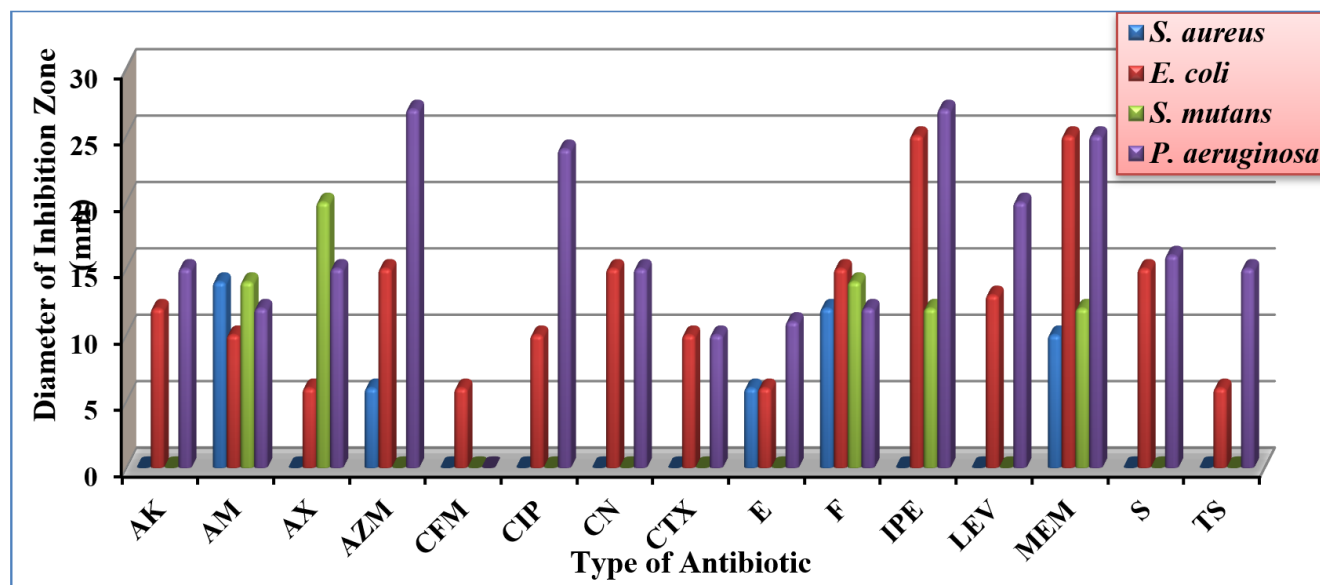


Fig. 14 Histogram of antibiotics activity according to diameters of inhibition zones

4 CONCLUSION

Pulsed laser ablation is a promising technique for synthesizing nanoparticles because of its simplicity, rapid processing, and relatively low cost. It also offers the possibility of controlling nanoparticle properties according to the intended application. Among the main ablation parameters, laser wavelength and pulse number are particularly important for controlling laser output and nanoparticle formation.

Under the present experimental conditions, the shorter laser wavelength produced nanoparticles with relatively smaller particle, crystallite, and grain sizes, as well as higher average absorbance, compared with those synthesized using the longer wavelength. In addition, increasing the number of laser pulses generally increased the absorbance and broadened the spectral peak. Based on the results of this work, suitable wavelength and pulse-number conditions can be selected for nanoparticle synthesis by laser

ablation.

The synthesized iron oxide nanoparticles showed promising structural and optical characteristics, which were reflected in their antibacterial activity. These findings suggest that Fe_3O_4 nanoparticles prepared by pulsed laser ablation may serve as potential supportive antimicrobial agents and could be further investigated for biomedical applications.

Author contributions

S.N.R.: design, implementation, writing and analysis of study data; A.S.J.: supervision and review; K.A.A.: design, supervision, and review; S.Z.K.: Review the arrangement and language of the manuscript.

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DECLARATIONS**Conflict of interest**

The authors declare no competing interests

Consent to publish

N/A

Ethical approval

N/A

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