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Synthesise of Iron Oxide Nanoparticles IONPs by Laser Method as an Antibacterial and Hemocompatibility Agent

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A B S T R A C T

In this work, room temperature laser ablation with an iron target in water was used to create iron oxide nanoparticles (IONPs) with a different number of pulses (100, 200, 300, 400 and 500) at a constant energy of 200 mJ. The colloidal solutions of the IONPs were studied and the effects of the number of pulses on the properties were investigated by Fourier transform infrared spectroscopy (FTIR), field emission scanning electron microscopy (FESEM), ultraviolet-visible spectroscopy (UV-VIS) and photoluminescence (PL). The FTIR spectra showed that the synthesised IONPs were formed, and the peaks appeared between (500-600) cm^{-1}. FESEM images showed that the IONPs have hemispherical structures and become spherical with increasing laser pulses. They also exhibited a small aggregation due to electrostatic forces. The UV-VIS results showed that the IONPs had an absorption shoulder at 300-400 nm, which increased with the laser pulses. The PL spectra of the IONPs showed strong, sharp peaks in the UV region at 370 nm, the intensity of which increased with increasing pulse duration, while the density of the nanoparticles in the solution increased. In addition, the antibacterial activities were evaluated using an agar well diffusion assay against Pseudomonas aeruginosa (P. aeruginosa), Staphylococcus aureus (S. aureus), Streptococcus mutans (S. mutans) and Acinetobacter baumannii (A. baumannii). The result showed that the IONPs have good antibacterial activity, which increased with the laser pulses due to the increased concentration of IONPs. A hemolysis and in vitro toxicity test also evaluated the compatibility with human blood on red blood cells.

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1. Introduction

Nanostructure materials are very interesting materials owing to their excellent properties like chemical, optical, electrical, biological, and mechanical features, which, compared to the base material due to the quantum effects and extreme ratio of surface area/volume, therefore they are appropriate for use in different applications area including solar cell, antibacterial, photodetector, batteries, catalyst, etc. [1-4]. These nanomaterials can be prepared

using various techniques and found in multiple forms, such as dots, wire, tubes and flowers related to preparation conditions; they can also be produced as pure metals, composites and metal oxides [5-10]. Iron oxide nanoparticles (IONPs) are among the nanomaterials that have resulted in an extreme concern in the study of nanotechnology because of their distinct magnetic, electronic and chemical characteristics, as well as their surface reactivity and biocompatibility. These significant features make them suitable for applications in biomedicine, the environment, energy storage and catalysis. Iron oxide nanoparticles (IONPs) have emerged as highly effective antibacterial agents, showing potential compared to standard antibiotics [11-14]. Promoting innovative antimicrobic policies is imperative, with the appeal of antibiotic-resistant bacteria establishing a substantial risk to community health. Iron oxide nanoparticles (IONPs), particularly in the forms of magnetite (Fe_3O_4) and maghemite (γ-Fe_2O_3), possess potent antibacterial properties attributed to their exceptional physicochemical characteristics and mechanisms of action [13]. These nanoparticles can be prepared through several approaches, such as Chemical Co-Precipitation, Thermal Decomposition, Hydrothermal Synthesis and laser ablation in liquid (LAL); this is a relatively novel method in which a laser is used to ablate a target that is submerged in a solution [14-18]. In general, the pulsed LAL process is a pioneering and flexible scheme for producing altered types and shapes of nanoparticles depending on laser parameters such as energy, wavelength, and pulse durations. This process has achieved considerable recognition because it can produce high-purity nanoparticles with controlled size, composition, and shape. Pulsed laser ablation in liquid (PLAL) involves interaction between a high-energy laser and a target immersed in a solution medium, leading to the formation of nanoparticles. This process has advantages over other traditional methods: simple and straightforward, extremely pure production without additional solvents, and eco-friendly process [17-20]. PLAL operates based on several fundamental physical methods, including Laser-Material Interaction, Plasma Development, NP Creation, and Liquid Medium Impacts. It has remarkable features compared to traditional methods for its effortlessness, changeability, deliberate production, eco-friendliness, high transparency, and the high feature of the developing nanoparticles [19-26]. Accordingly, PLAL was employed to manufacture iron oxide nanoparticles (IONPs) and estimate their categorization using different approaches such as FTIR, FESEM, UV-VIS, and PL. Also, the antibacterial activity versus some strains of microorganisms and biocompatibility via hemolysis tests on human red blood cells were investigated.

3. Experimental Procedure

A 99.99% (Sigma-Aldrich) pure iron sheet was laboured as the object's raw material for the ablation process. The ablation process of the iron target in water utilising a nanosecond Nd: YAG laser (1064nm, 9ns, & 10Hz), the beam was motivated via utilising a 120 mm positive lens. The process used several laser pulses adjusted from 100 to 400 pulses, with fixed laser energy at 200 mJ. The prepared nanoparticle features were done using Fourier-transform infrared spectroscopy (8000 Series, Shimadzu, Japan) in the range of 400–4000 cm−1 to investigate the chemical composition of iron oxide nanoparticles IONPs. The synthesised nanoparticle morphology was analysed utilising field emission scanning electron microscopy (Tesca, MIRRA ШI, Czech). The optical assets were approved by managing an 1800 Shimadzu (Japan) spectrophotometer and photoluminescence spectroscopy (Shimadzu RF-551, Japan).

The activity of IONPs against A. baumannii, P. aeruginosa, S. mutans, and S. aureus was inspected using an agar-well diffusion method. The bacteria were sub-cultured on brain heart blend agar. A culture medium was inoculated using a bacterial suspension equal to a 0.5 McFarland standard, corresponding to a microbial concentration of approximately $5x10^7$ CFU/mL, and left for 15 minutes for absorption. Following this, a sterile 5 mm pipette was employed to create wells in the agar, and 70 µL of IONPs at different concentrations (600, 1000, 1300, and 1600 µg/mL) was added to each well. Simultaneously, water was utilised as the control. Following 24 hours of incubation at 37°C, the inhibition zones were measured by evaluating the width of the clear areas around the wells using a ruler.

The hemolytic activity of IONPs was also examined. Fresh human blood samples were collected from healthy volunteers into a gel, and EDTA tubes, and 1600 µL of normal saline was mixed with 200 µL of the separated blood. Next, 200 µL of the diluted blood was added to the nanoparticle solutions. After incubating for 1 hour at 37°C, the samples were centrifuged at 700 rpm for 5 minutes [20]. Afterwards, measuring the absorbance at 541nm, and the fraction of hemolysis was estimated using the formula:

$$Hemolysis\ (\%) = \frac{OD_s - OD_n}{OD_p - OD_n} * 100 \qquad (1)$$

ODs is NPs' optical density, ODn is a negative control, and ODp is a positive control. This experiment was conducted according to the Food and Drug Administration guidelines, the National Institute of Health, and the Helsinki Declaration and Regulation of 1975, which serves as a statement of ethical standards for treating experimental subjects. The Department of Applied Sciences, University of Technology, Baghdad, Iraq's institutional ethical committee granted the authorization, which was sanctioned after getting approval from the hospitals in the medical city (Ref. No. 4832 ASBT 8/11/2021).

4. Results and Discussion

Fig. 1 demonstrates the FTIR spectrum of iron oxide nanoparticles (IONPs) synthesised with varying pulses (100, 200, 300, 400, and 500) at a laser energy of 200 mJ. The spectrum shows a band between (3200 and 3380) cm-1 according to the O-H vibrational mode. The highest peak was at ~1639cm-1, which is attributable to hydroxyl groups. While peaks between (500-600) cm-1 were associated with the vibration mode of Fe-O [27,28]. Therefore, evidence of the formation of IO NPs was established on the results acquired from the FTIR investigation. Also, no significant peaks were detected for the impurities.

Fig. 2 indicates the FESEM images show iron oxide nanoparticles (IONPs) synthesised through pulsed laser ablation in water, with laser beam energy set at 200 mJ and pulse numbers ranging from 100 to 500. The morphology of the IONPs was semi-spherical, with aggregation observed due to electrostatic forces [29,30]. **Fig. 3** The UV-VIS spectrum of IONPs prepared with varying numbers of pulses at 200 mJ reveals that the maximum absorption intensity increases with multi-laser pulses. This trend is correlated with the increase in nanoparticle concentration. Additionally, the absorbance spectra of iron oxide nanoparticle (IONP) suspensions do not exhibit the characteristic plasmon resonance typically associated with noble metals. This absence complicates the spectroscopic detection and identification of nanoparticles in the liquid. While exhibiting an absorption shoulder near 300-400 nm, these properties are due to nanoparticles larger than 20 nm [31,32].

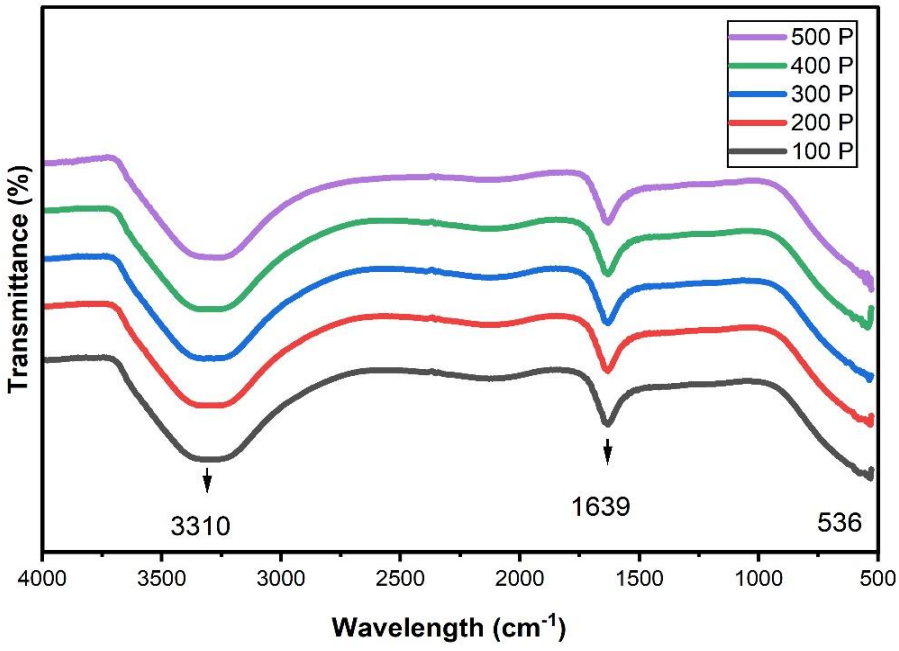

Figure 1: FTIR spectra of iron oxide nanoparticles IONPs prepared via PLAL @ 200mJ with altered pulses of a) 100, b) 200, c) 300, d) 400, and e) 500.

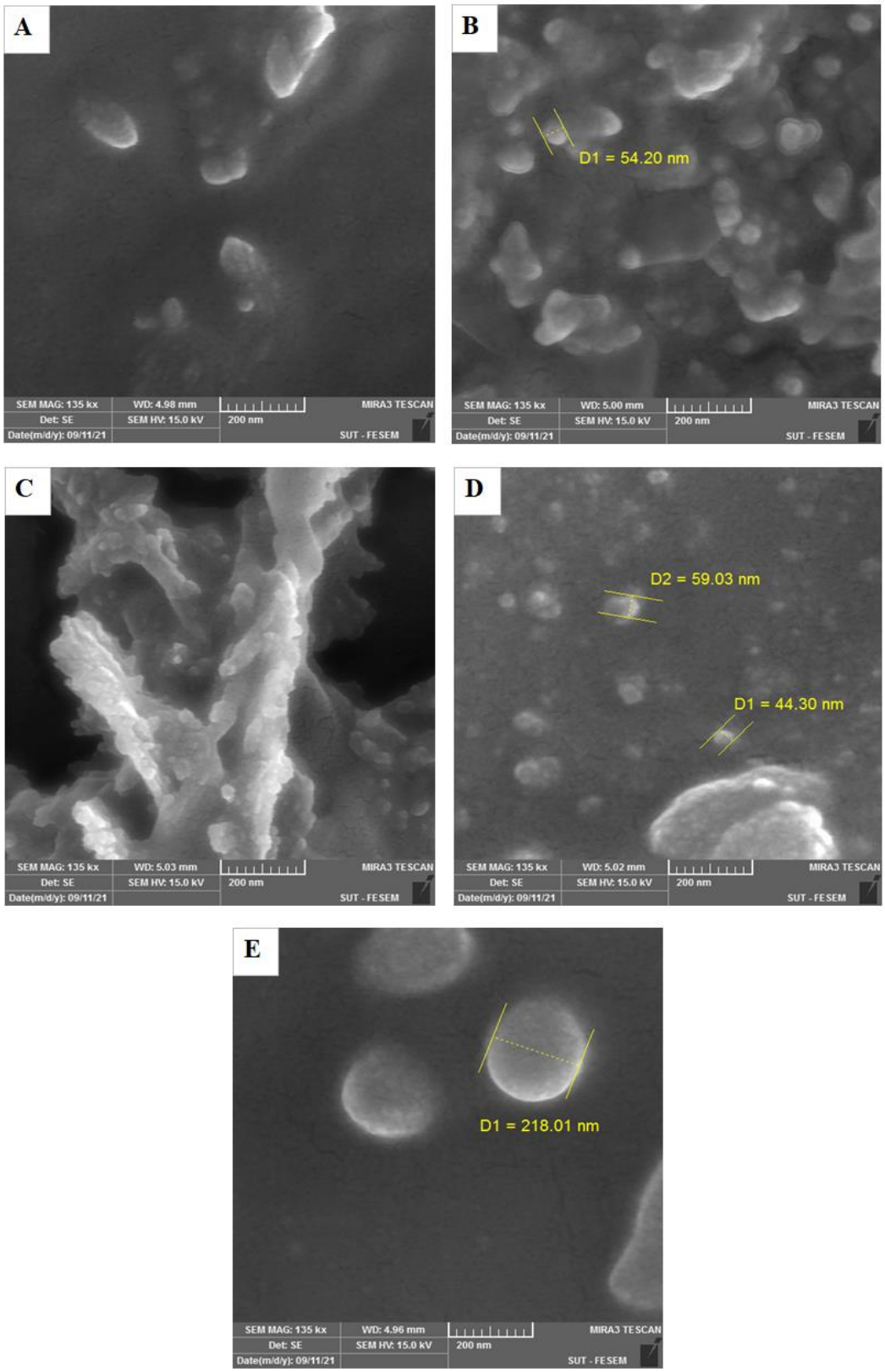

Figure 2: FESEM images of IONP prepared @ 200mJ with altered pulses of: A) 100, B) 200, C) 300, D) 400, and E) 500

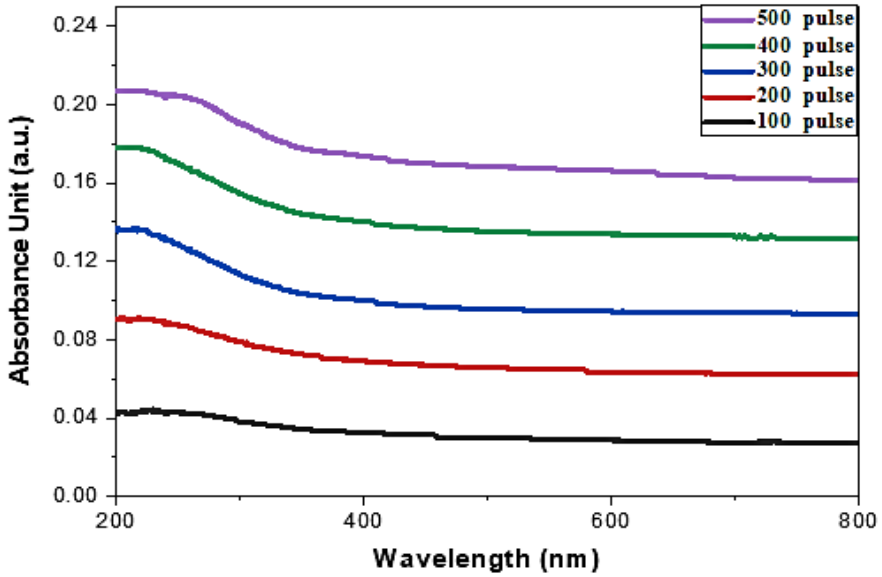

Figure 3: An absorbance spectrum of iron oxide nanoparticles (IONPs) prepared by PLAL @ 200mJ with different pulses.

Fig. 4 shows the PL spectrum of forming iron oxide nanoparticles IONP using laser ablation of the iron target in water at 200 mJ with several laser pulses ranging from 100 to 500. These spectra show PL maximum intensity around 370 nm. This intensity increases with multi-laser pulses, revealing a higher particle density in the solution medium. In iron oxide nanoparticles (IONPs), the minimal exciton level is observed due to excitation between the valence band (VB), which includes a mix of Fe(3d) and O(2p) levels, and the conduction band (CB), primarily formed from Fe(4s) levels. When excitation occurs, an electron is promoted from the VB to the CB, creating a hole (h) in the VB. The exciton, comprising the excited electron (e) and the hole, moves easily between the bands, and recombining these components results in emission at 370 nm, which is observed as red emission [11,12].

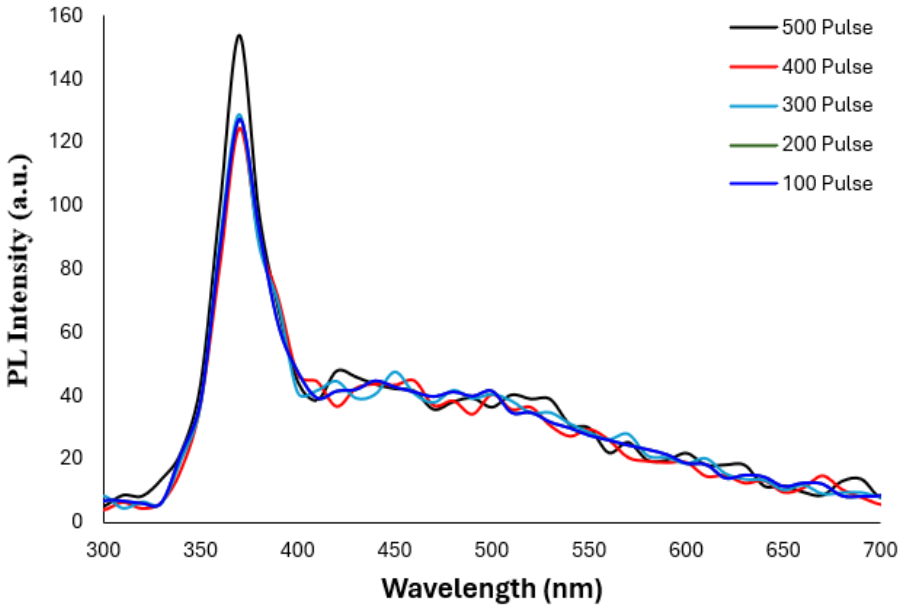

Figure 4: PL band of iron oxide nanoparticles (IONPs) prepared by PLAL @200mJ with different pulse numbers.

Fig. 5 displays the antibacterial activity of IONPs with different concentrations (600, 1000, 1300, & 1600) µg/mL against the four types of microbes. Iron oxide nanoparticles (IONPs) exhibit good activity against all pathogens,

which increases as the concentration of NPs increases. ROS causes modifications to macromolecules through oxidative stress, leading to the formation of short-lived and highly reactive free radicals. These radicals can disrupt nuclear stability and overall cell health, resulting in cell death [30, 33].

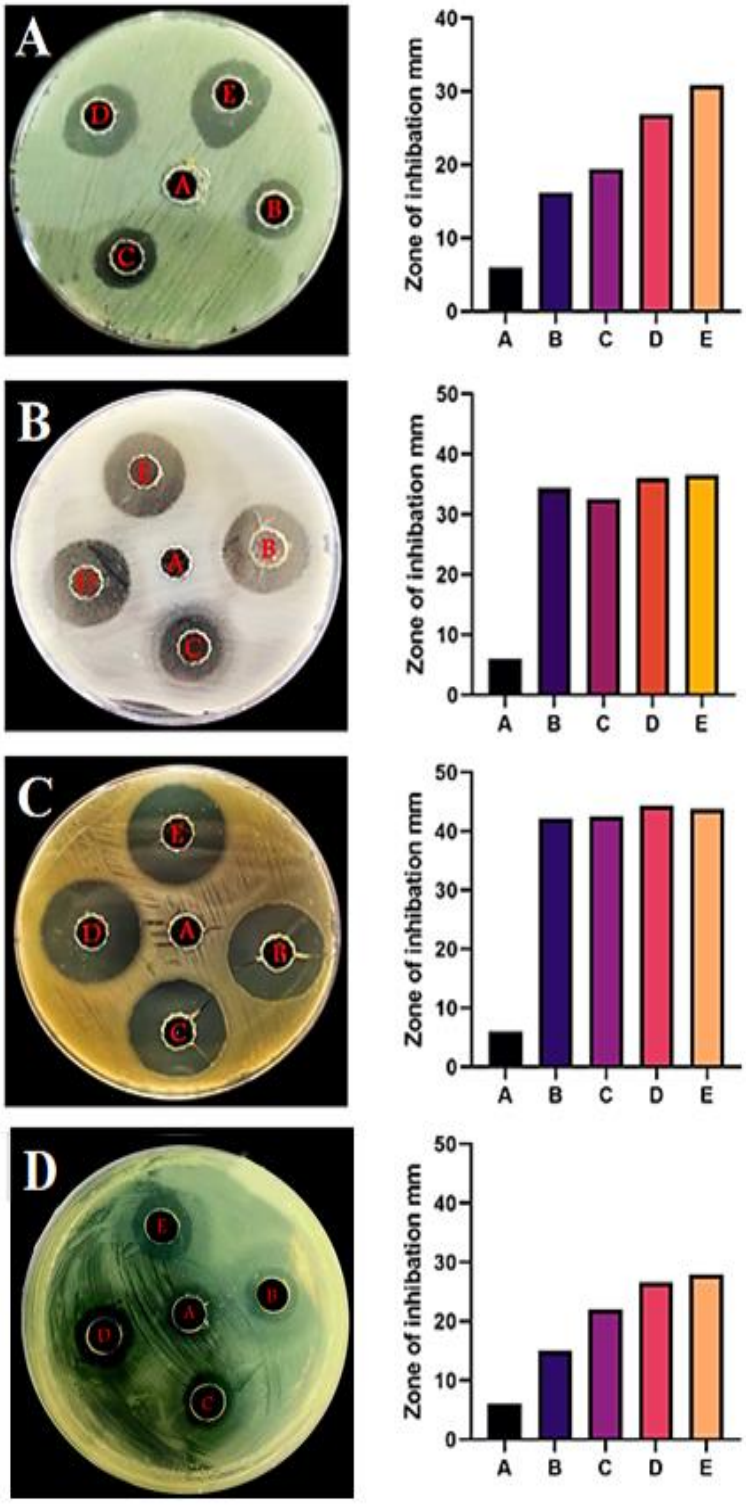

Figure 5: Antibacterial activity of IONPs prepared @ 200mJ/500pls anti A) P. aeruginosa., B) S. aureus., C) S. mutans, & D) A. baumannii. A) control, B) 600µg/mL, C) 1000µg/mL, D) 1300µg/mL, and E) 1600µg/mL.

Fig. 6 described the percentage of human RBCs hemolysis in various concentrations of IONPs, where hemolysis was found to be 2.35, 3.76, 4.32, 16.96 and 58.5(%) at (600, 1000, 1300, and 1600) µg/ml concentration respectively, which seen the percentage of hemolysis is dependent on the concentration of iron oxide nanoparticles. The results revealed that iron oxide nanoparticles (IONPs) exhibit low hemolytic activity (less than 5%) at lower concentrations (600-1300 µg/mL). However, this activity increases at higher concentrations, reaching a notable level at 1600 µg/mL. These findings suggest that IONPs have appropriate biocompatibility at lower concentrations but may cause red blood cell destruction at higher concentrations. This information is crucial for determining safe and effective dosages for potential medical applications of IONPs.

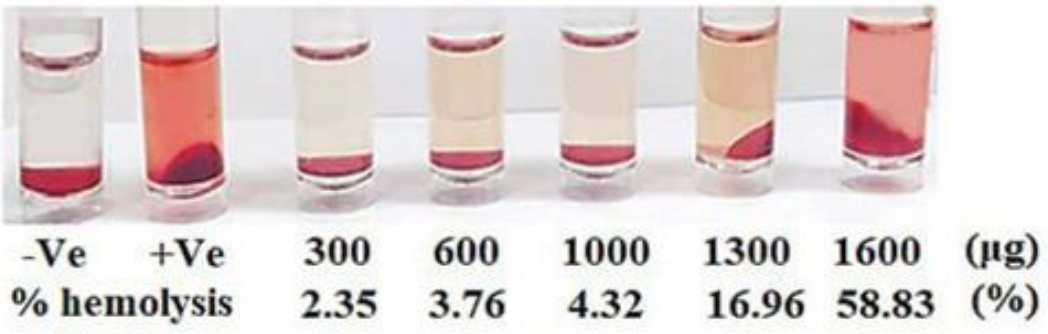

Figure 6: Hemolysis of human RBCs treated with IONPs at different concentrations.

5. Conclusions

Iron oxide nanoparticles (IONPs) are synthesised in a single step by pulsed laser ablation in the nanosecond range in liquid with a varying number of laser pulses. Characterization by various measurements such as FTIR, FESEM, UV and PL confirm the formation of iron oxide nanoparticles (IONPs) without any impurities or additional solvents. Furthermore, the result shows that the synthesised iron oxide nanoparticles IONPs exhibited promising antibacterial activity against pathogens such as P. aeruginosa, S. aureus, S. mutans and A. baumannii. The antibacterial activity increased with increasing concentration of the nanoparticle NPs, which was attributed to the deflection of cell tissue and the formation of reactive oxygen species (ROS). In addition, the researchers discovered a concentration-dependent hemolytic effect on human red blood cells, highlighting the need to adjust IONP concentrations for biomedical applications. The conclusions emphasise the promise of IONPs as efficient antibacterial agents and focus on their suitability for various medical applications.

Conflict of Interest

The authors declare that they have no conflict of interest.

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