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Study and Synthesis of Fe_3O_4 Nanoparticles Prepared by Laser Ablation and its Biomedical Application

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Abstract: This study aimed to determinate the antibacterial activity and cytotoxic effects (Fe_3O_4 NPs). Antimicrobial activity against pathogenic microbes which obviously indicates that Fe_3O_4 NPs are considered an valuable antimicrobial agents. The (G +ve bacteria) *S. aureus* were more susceptible to iron oxide nanoparticles when compared to the *E. coli* and *P. aeruginosa* (G -ve bacteria). Results indicate that inhibitory activity of iron oxide nanoparticles increases with increase of the Fe_3O_4 NPs concentrations. Fe_3O_4 NPs was not effect on every cells but through an suitable magnetic field of iron oxide as for the detection of the toxicity of nanoparticles it was observed that it had toxicity to red blood cells through the complete haemolysis. The current study concluded the Fe_3O_4 NPs can effects on the inflammatory response that which cause the oxidative stress and may negatively affect the cellular function and Fe_3O_4 NPs in high concentrations have a hemagglutinated.

Key words: Fe_3O_4 NPs, laser ablation synthesis, antibacterial activity, cytotoxicity assay, inflammatory, hemagglutinated

INTRODUCTION

In recent years research has increased in the subject of nanometer-size metallic nanoparticles because it occupies a middle between bulk materials and atoms (Iglesias-Silva *et al*., 2007). Iron oxide has taken an important place among the other nanoparticles due to its diverse scientific and technological applications like biosensor (Berry and Curtis, 2003) antibacterial activity (Babes *et al*., 1999) food preservation (Chan *et al*., 1993) magnetic storage medium, ferro-fluids, magnetic freezing, magnetic resonance imaging, hypothermic tumer treatments, cell sorting and targeted drug delivery (Beets-Tan *et al*., 1998; Gupta and Gupta, 2005; Iida *et al*., 2007). In addition to its enormous application in medicine filed may be because own biocompatibility of it and magnetic characteristic (Matheson and Tratnyek, 1994). Synthesis of NPIOs are carried out by different chemical approaches like co precipitation, sol gel and compulsory hydrolysis, hydro-thermal, surfactant mediated/pattern creation, micro-imulsion, electro-chemical and laser pyrolysis. Amongst these the co-precipitation method is may be the easiest and most professional chemical lane during which a superior quantity of nanoparticles be able to be created (Yao *et al*., 2009). Due to emergence for high resistance to antibiotics by bacteria it became necessary to develop bactericidal from different sources (Mahdy *et al*., 2012). Modern development in nanotechnology field has provided gorgeous method for created another antimicrobial agents and inhibited the biofilme formation (Mohapatra and Anand, 2010). Nanoparticles have a broad spectrum effect on pathogenic organisms (Mahdy *et al*., 2012; Tran *et al*., 2010) but the information on the toxicity of nanoparticles is low, hence, the aim of this study to identify the toxicity of iron oxide. Because of the small size of nanoparticles and paramagnetic properties this help it to direct interaction with microorganisms, causing either damage of cell wall or disruption of metabolic functions, leading to cell destruction. Oxidative injure and haemolysis caused by ROs have a main function to progress of diseases like thalassemia, glucose-6-phosphate dehydrogenase deficiency and sickle cell anaemia. Red blood cells are the first targets of ROs because their membranes contain a high proportion of fatty acids which are strong promoters of ROs. Oxidation reduces the protein content of membranes, deformation of the red blood cells and disturbs

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microcirculation (Furno *et al*., 2004; Yang *et al*., 2006; Rice-Evans *et al*., 1986 and Yu, 2001) as well its responsible on decomposition of blood (Flynn *et al*., 1983). Blood decomposition was based on evidence of damage caused by free radicals and counteraction by antioxidants. It is helpful to showing the oxidizing or antioxidising agents (Ko *et al*., 1997).

MATERIALS AND METHODS

Fe_3O_4NPs preparation: The pure Fe goal with 99.99% was used initially set on the down of a plastic vessel then dissolved with DW and ultrasound for 4 h then the Fe produced through a 0.22 μm filter. Finally, Fe target synthesized was treated with post-laser under Nd:YAG laser of 1064 nm wavelength and by 350 mJ/pulse energy was used for target ablation and the ablation time was 20 min to obtaining smaller size of Fe_3O_4 NPs.

Characterization Fe_3O_4 NPs: Achieved in the division of applied science, university of technology and included UV (Metertech, SP8001 spectrophotometer, Japan) was employed for the visual test of Fe_3O_4 NPs within the 200-800 nm spectral range, SEM for collimated and alert beam of high energy electrons to create images from a sample's surface and FTIR.

Antimicrobial activity assay: Study of antibacterial activity by well-diffusion assay. Antibacterial activities of Fe_3O_4 NPs were done for *E. coli, P. aeruginosa* (G -ve) and *S. aureus* (G +ve) bacteria. The 100 μL from fresh culture having 10^6 CFU/mL of each pathogenic bacteria was spreading on nutrient agar plates. Plates were left to dry for ten min. Formerly 6 mm wells were punched into agar plates. The 100 μL of Fe_3O_4 NPs suspension was poured onto wells. Incubation at 37°C for 24 h, the diameter of zone of inhibition were measured.

Anti-haemolytic assay: This analyze is helpful for showing the agents that have an oxidising or antioxidising action (Costa *et al*., 2009). Hydrogen peroxide have all been broadly studied in the biological membranes (Ebrahimzadeh *et al*., 2009a, b, 2010; Nabavi *et al*., 2011). Nanoparticles antihaemolytic activity for this study be detected according to with some modified. human blood were concentrated in phosphate buffer to 4% suspension. Nanoparticles were set in phosphate buffer at (0.25, 0.5, 1, 2 and 4) mg/mL^{-1} concentrations. Then 2 mL of red blood cell suspension added for 1 mL of nanoparticles in each concentration and enough phosphate buffer to reach the final volume to 5 mL. Incubation at room temperature for 5 min after that 0.5 mL of 0.3% H_2O_2 was added to encourage oxidative degradation of membrane lipids and the combination was shaken for 30 min. Centrifuged at 1500 g to the sample for 10 min and the resulting supernatant was removed and used to estimate their haemolytic act by measured the absorbance at 540 nm wavelength. Haemolysis in the presence nanoparticles was calculated by compared with control (Ebrahimzadeh *et al*., 2009a, b; Djeridane *et al*., 2007). Antiyhaemolysis ratio was calculated as follows:

$$\text{Antihaemolysis} = [(A_0 - A_1)/A_0] \times 100\%$$

A_0 that refers to the absorbance of control (hydrogen peroxide with red blood cell without nanoparticles) was considered as 100% haemolytic activity whil the A_1 was refers to the absorbance with the nanoparticles. Each experiments was performed in triplicate.

RESULTS AND DISCUSSION

Ultraviolet-visible spectrophotometer: The UV-visible spectrophotometer was carried out to investigate the optical properties of Fe_3O_4 NPs colloidal prepared by laser ablation in deionized water it is very important to give information about the absorption edge, optical band gap between the electronic transitions, absorption coefficient, etc. Figure 1 illuminated the transmittance and absorbance curves as a function of incident wavelength of samples prepared at 1062 nm laser wavelength at constant laser energy (350 mJ) laser pulses.

Scanning Electron Microscope (SEM) results: The surface morphology of the obtained Fe_3O_4 NPs synthesized at 300 laser pulses is observed by Scanning Electron Microscope (SEM) as shown in Fig. 2.

FT-IR spectroscopy: FT-IR spectroscopy FT-IR spectrum of the synthesized Fe_2O_4 NPs showed in Fig. 3. FTIR spectra of metal oxide Fe_2O_4 NPs showed vibrations in the region 400-600 cm^{-1} which refer to vibrations M-O (M = Fe) that confirm the configuration of Fe_2O_4 NPs. In Fe_2O_4 NPs, the peak showing at 1632 cm^{-1} can be refer to the angular deformation of water δH-OH while the peak showing at 3436 cm^{-1} can be appearing to the O-H stretching of water. Peak at 3495 cm^{-1} is assigned to adsorbed water that gives rise to the stretching form of hydroxyl and the bending mode of hydroxyl at 1626 cm^{-1}.

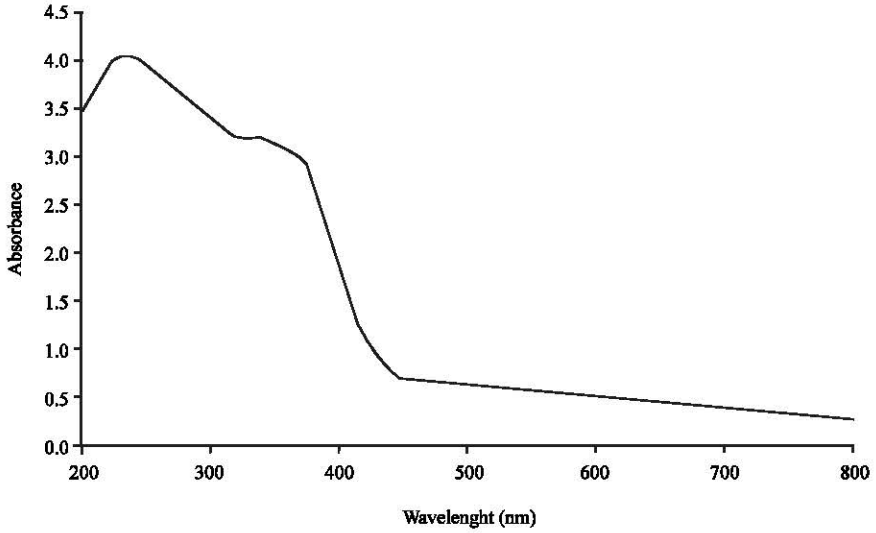

Fig. 1: Ultraviolet-visible spectrophotometer of Fe_3O_4 NPs produced by pulsed-laser ablation of Fe target in double distilled water at a laser energy 350 mJ/pulse, 20 min

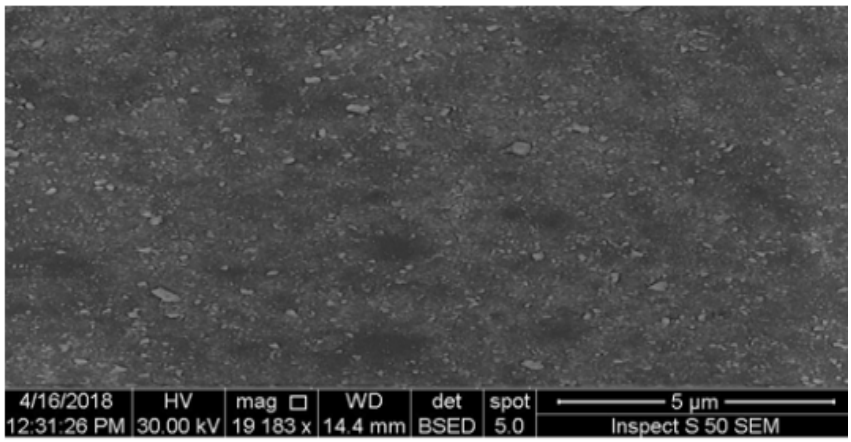

Fig. 2: SEM of Fe_3O_4 NPs produced by pulsed-laser ablation of Fe target in double DW at 350 mJ/pulse a laser energy for 20 min

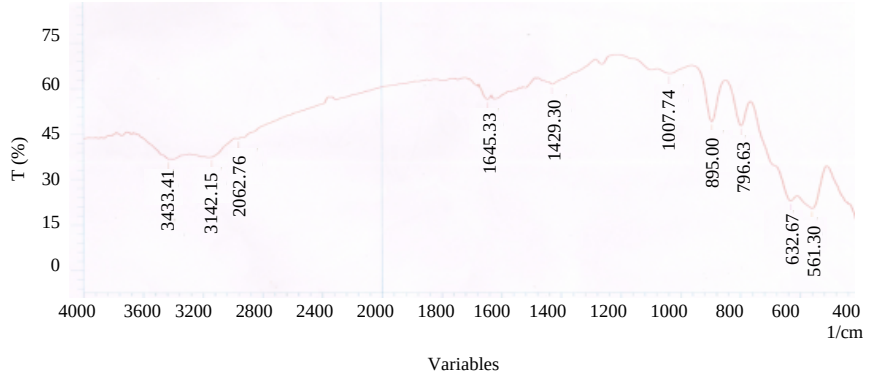

Fig. 3: FT-IR spectroscopy of Fe_3O_4 NPs produced by pulsed-laser in the range of 400-4000 cm^{-1}. The FTIR results show the high purity of the obtained Fe_3O_4

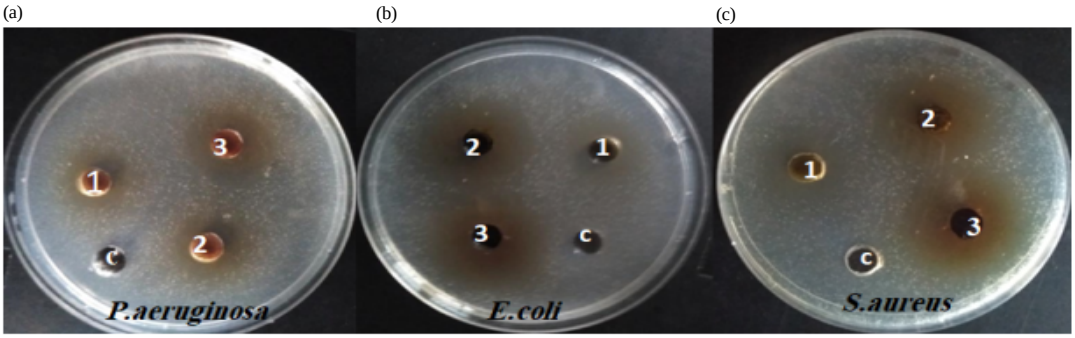

Fig. 4a-c): Antibacterial activity of Fe_3O_4 NPs prepared by using pulsed-laser against some pathogenic bacteria; c) represent control; 1) Concentrations 250 μg/L; 2) Concentrations 500 μg/mL and 3) Concentrations 750 μg/mL

Table 1: Antibacterial activity of iron oxide nanoparticle against *S. aureus*. *E. coli* and *P. aeruginosa*; c) Represent control; 1) Concentrations 250 μg/mL; 2) Concentrations 500 μg/mL and 3) Concentrations 750 μg/mL

	Zone of inhibition (mm) Fe_3O_4 NPs			
Microorganisms	c	1	2	3
S. aureus	-	14.33	19.66	22.33
P. aeruginosa	-	12.00	16.33	18.33
E. coli	-	8.00	11.00	15.66

Another peaks are as follows: 913 cm^{-1} to put vibratory water from the water molecule 0.566 cm^{-1} means the extension of FeO of hematite and 482 cm^{-1} to put the lattice FeO_6. The present results correspond to the values mentioned in the available literature (Arshad *et al*., 2011; Jagminas *et al*., 2002; Jagminas *et al*., 2004; Zhang *et al*., 2006; Ansari *et al*., 2011).

Antibacterial activity: Antibacterial activity Fe_3O_4 NPs showed antibacterial activity against both G -ve and +ve bacteria which clearly indicates that these nanoparticles are effective antibacterial agents. Figure 4 and Table 1 various concentrations of Fe_3O_4 NPs were shown. The diameter of inhibition zones around each well is represented in Table. The G +ve bacteria *S. aureus* were more susceptible to iron oxide nanoparticles by zone of inhibition reached to (22.33 mm) when compared to the G -ve bacteria *P. aeruginosa*, finally in *E. coli* (15.66 mm). According to the results we observed when increasing the concentration of the nanoparticle this due to increases the antibacterial activity. Bacterial inhibition depends on concentration. Fe_3O_4 NPs do not damagingly effect on all cells while through an suitable magnetic field of Fe_3O_4 NPs may be used or help to damage bacteria Taylor and Webster (2009a, b). According to Lee *et al.* (2008a, b) the Fe_3O_4 NPs affected the inactivation of bacteria through the diffusion of the small particles ranging from 10-80 nm into bacterial membranes.

Antihaemolytic activity: The size, shape, charge, surface area and assembly of IONPs have a significant impact on the toxicological study which is due to the strong electrostatic reaction between the positively charged cell surface and negatively charged IONPs at high doses (Cai *et al*., 2013). The cause of hemagglutination of blood is due to the generation of free radicals whose reaction or association with biomechanical molecules such as lipids, DNA and proteins leads to swelling and rupture of cells (Al-Akhras and Grossweiner, 1996). Nanoparticles have the ability to strongly attract and weaken the cell and this high attraction forces the red blood cells to attract together and in very large numbers which leads to a significant accumulation of blood (Loeb *et al*., 2010). Of the iron oxide nanoparticles have toxic effect in a high concentration (Aula *et al*., 2014) while they cause hemagglutination effect at high concentrations (Khalil *et al*., 2017).

Nevertheless, it should also be noted that the bacterial G -ve strains of *E. coli* and *P. aeruginosa* contain lower-area inhibitory sizes than G +ve bacterial strains of *S. aureus*. This observation may also be a sign of increased G -ve resistance/tolerance of these nanoparticles to G +ve bacteria (Premanathan *et al*., 2011). A number of researchers have shown that the little size of NPs be able to also add to bactericidal effects. The inactivation *E. coli* by zero-valent iron NPs could be due to penetration of small particles sizes ranging from (10-80 nm) in the *E. coli* membranes (Lee *et al*., 2008). The zero-iron nanometer reaction with oxygen inside the cells this causes oxidative stress and ultimately lead to interruption the cell membrane (Makhluf *et al*., 2005; Zhang *et al*., 2007). It is also important to note that the NPs of Fe_3O_4 do not negatively affect all cells and therefore, it can be argued that with an appropriate external magnetic field, Fe_3O_4 NPs may be directed to kill bacteria as needed throughout the body (Taylor and Webster, 2009) (Fig. 5).

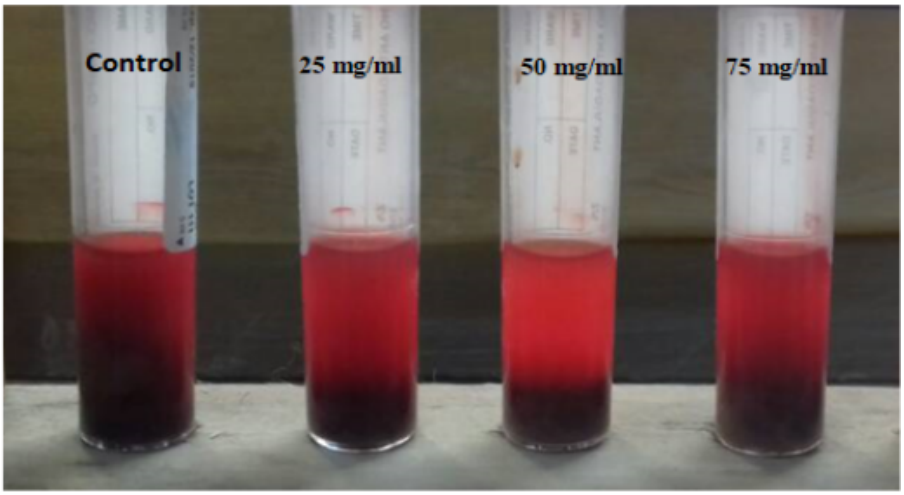

Fig. 5: The toxicity effect of Fe_3O_4 nanoparticles on human red blood cells

CONCLUSION

Nanoparticles toxicity on RBCs is a product of a mixture of shape and component. The effect of shape is beginning on the degrees of free rotation. The effects of composition are founded on the attraction for the nanoparticles to itself and to the red blood cells. The larger the nanoparticles attraction to agglomerate to itself lacking precipitating out of the solution, the more hemolytic activity will be detected. Fe_3O_4 NPs in high concentrations have a hemagglutinated either in the few concentrations does not have any toxic effect.

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