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Ultrasound-Assisted Synthesis of Iron Oxide Nanoparticles: Application in Cytoxocity and Antibacterial Activity

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Abstract

At the present time, the phenomenon of antibiotic resistance has increased by different species of bacteria. In this way, particularly in the situations of Metal Nanoparticles (MNPs) fabrication and MNPs surface modification, the emergence of nanotechnology as a new weapon has drawn increased attention. Currently, the safe way to manufacture nanoscales is at the lowest possible cost and the least harm to the environment of Fe_2O_3 NPs with novel shape through Ultrasound assisted. Ultraviolet Visible Spectrophotometer (UV-Vis), Energy Dispersive X-ray spectroscopy (EDX), Scanning Electron Microscopy (SEM), Atomic Force Microscopic (AFM), X-ray Diffraction (XRD). These techniques were applied for physical characterization. Disc diffusion assay and Minimum Inhibitory Concentration (MIC) (16 µg/ml), were evaluated against gram negative (*P. aeruginosa, Klebsiella spp.*) and gram positive (*S. aureus, S. pyogenes*) Fe_2O_3 NPs with an average diameter size of 30 nm. Where the activity of iron nanoparticles prepared by a physical method showed a distinct activity against selected cancer cells.

Keywords: Fe_2O_3 NPs • UBC-40 cells • MRSA • Scanning electron microscopy

Introduction

Nanotechnology is the creation of materials and devices by controlling of matter at the levels of atoms, molecules and supramolecular (nanoscale) structures, is the use of very small particles of materials to create new large scale materials. Better understand difference among various scales [1]. Antibiotics used to treat infectious illnesses are manufactured worldwide in around 100,000 tons per year. Antibiotic overuse has caused pathogenic strains, particularly in bacteria, to become multidrug resistant [2]. Iron Oxide Nanoparticles (IONPs) have drawn a lot of interest because of their distinctive characteristics. Greater surface area, super para magnetism, surface to volume ratio and simple separation techniques. Were determined various physical, chemical, and biological methods have been adopted to synthesize magnetic NPs with suitable surface chemistry. Iron oxides exhibit great potential in the fields of life sciences such as biomedicine, agriculture, and environment. Applications of magnetic NPs can be made more biocompatible and nontoxic by covering a specific surface with organic or inorganic molecules, such as surfactants, medications, proteins, starches, enzymes, antibodies, nucleotides, nonionic detergents and polyelectrolytes [3]. Nanoparticle toxicology is a developing area that focuses on establishing the hazard of nanoparticles, the result of their potential risk, in light of their growing used and exposure likelihood [4]. The study of nanotoxicology is becoming more important branch of nanotechnology. The term "nanotoxicology" refers to the study of how nanostructures interact with biological systems. The focus on determining how the physical and chemical characteristics of nanostructures (such as their size, shape, surface chemistry, composition, and aggregation) related to the induction of toxic biological responses [5]. Nanomaterial's applications in many fields such as environments and public health's have been grabbing considerable attention. Experiments on animal models have showed many toxic effects like inflammation, growth decreased rate as well as neurobehavioral changes. Great surface to volume ratio, chemical composition, size, dosage, and retention in the body represent the major factors that affect nanoparticles toxicity [6]. Despite the fact that IONPs have many beneficial applications in many medical fields. There are only a few studies that investigated their effects on the reproductive system. Therefore, uncover their effects on the reproductive system. The aim of present study was to reform the effect of IONPs on the cell line and against bacteria.

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Materials and Methods

Iron (III) chloride ($FeCl_3$) anhydrous, Iron Sulfate Heptahydrate ($FeSO_4.7H_2O$), Ethanoland Sodium Hydroxide (NaOH) pellts (2-3), beakers glass (100-200) mL, deionzed water and ultrasonocator (30 KHz, 20 W).

Synthesis of Iron Oxide Nanoparticles (IONPs)

To maintain the molar ratio 2:1, 0.05 mmol of $FeSO_4.7H_2O$ were made by dissolving in 250 ml of deionized water. However 0.1 mmol of $FeCl_3$ anhydrous were prepared in the same method ultra sonocator was placed to both salts aqueous solution in 100 ml round bottom flask with heating at 60 C-70 C 0.4M of aqueous NaOH solution was prepared which constantly added to the mixture drop by drop until the pH reached 11-13. The flask was covered with a cork, and the reaction was allowed to run at the specified settings for one hour. After the reaction the mixture was centrifuged at 6000 rpm for 5 minutes after being allowed to cool at Room Temperature (RT). However, the supernatant was throwed and kept the black precipitate. The precipitate was dried overnight at 40°C in an oven to get the dry powder (Figure 1) [7].

Figure 1. Synthesis IONPs powder.

Characterization of IONPs

Characterization and understanding nanoparticles and developing successful applications were developed. Methods were used to determine NPs characteristics: SEM and TEM to characterize the shapes and sizes. Atomic Force Microscopy (AFM) was used. UV-visible spectrophotometer then used to investigate the optical properties of the sample. (XRD) was a method used in materials research to identify material's crystallographic structure [8].

Determination of Minimum Inhibitory Concentration (MIC)

IONPs activities of bacteriostatic were measured by MIC assays [9]. An appropriate volume of bacteria (2 L) in Mueller Hinton broth MHB. The MHB of two fold dilution serials were added to MHB (64,32,16,8,4) µg/ml by using 6 tubes of MHB respectively . The tubes were incubated for 24 hours at 37°C. These inoculum of bacteria preparation at 0.1 M concentration was demonstrated an against bacterial test and assay the turbidity was observed in growth and non-turbidity as no growth after and the medium had been

These inoculum of bacteria preparation at 0.1 M concentration was demonstrated an against bacterial test and assay the turbidity was observed in growth and non-turbidity as no growth after and the medium had been incubated for 24 hours at 37°C. The lowest concentration was shown by the MIC values (Figure 2).

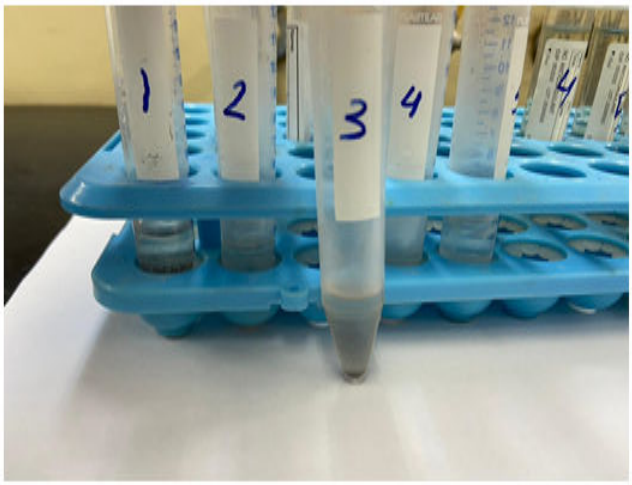

Figure 2. Clear fluid with no turbidity formation.

Multiple Drug Resistance bacteria (MDR bacteria)

Detection of gram negative (*Pseudomonas aeruginosa, Klebsiella spp.*) and gram positive (*S. aureus, Streptococcus pyogenes*) all strain were isolated from urine patients suffer Urinary Tract Infection (UTI) were employed to evaluate the IONPs' antibacterial performance. The microbiology lab of Baghdad university provided these strains. Finally, bacterial strains were maintained at 4°C on nutrient agar slants.

Disc diffusion method

Antibacterial activity was determined by using disc diffusion method [10]. For each test, overnight MHB cultures of a specific strain of bacteria were freshly made and inoculed applied using swabs to the surface of the solidified medium. IONPs were placed on discs at various concentrations between (64, 32, 16, 8, 4) µg/ml after the medium had dried for 10 minutes [11-13].

Results and Discussion

Cytotoxic by MTT assay

Evaluation of three concentrations of chemical IONPs anti-normal cell cytotoxic of HdFn and UBC-40 cells in 96-well plates, after 24 hours were made. IONPs was determined the equation of IC50 value: Cell viability=Ab S /Ab C _100 (Figure 3).

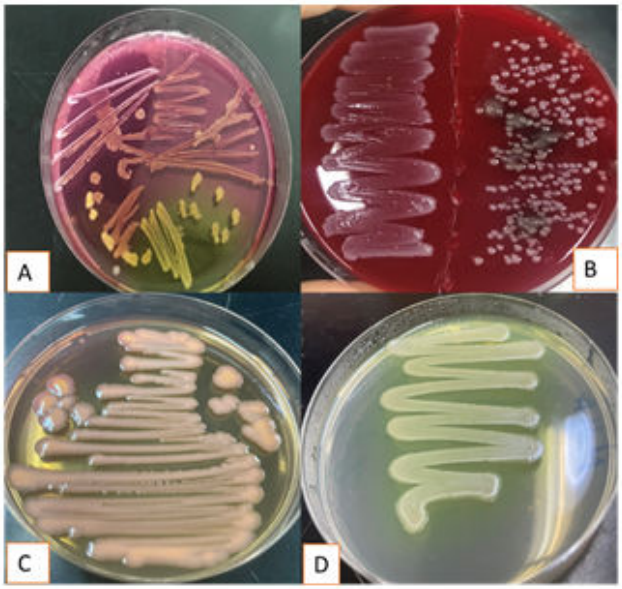

Figure 3. Bacteria isolation; A) *S. aureus*; B) *Streptococcus pyogenes;* C) *Klebsiella* spp; D) *Pseudomonas aeruginosa*.

Physicochemical properties of NPs

Ultraviolet-Visible (UV-Vis) spectrophotometer: UV-visible absorption spectrophotometer is widely used as a technique to examine the optical properties of certain nanoscale particles. The optical properties of the synthesized iron oxide nanoparticles by UV-vis spectrophotometer at room temperature is demonstrated (Figure 4). A broad peak has been found in 345 nm, which is a characteristic standard peak of spherical phase iron oxide, demonstrating that the synthesized products are pure iron oxide. This sharp peak shows that the particles are in nano size (Figure 4).

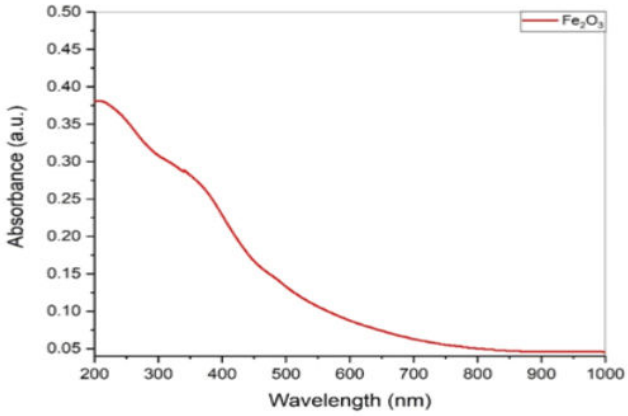

Figure 4. UV-Vis spectrophotometry of IONPs sonochemical.

X-Ray Diffraction (XRD): The XRD determination of crystallinity analysis and purity of Fe_2O_3 nanoparticles was applied comparing with XRD patterns. Fe_2O_3 NPs was demonstrated. The XRD spectra of nanoparticle powder was proven in figure revealed distinct peaks that corresponded to the diffraction peaks, where the grain (nanoparticles) is 33.64 nm. The IONPs was formed at (31.95), (35.8) and (45.6) which used to estimate the size of the crystallite by Debye-Scherrer equation of Fe_2O_3 NPs (Figure 5).

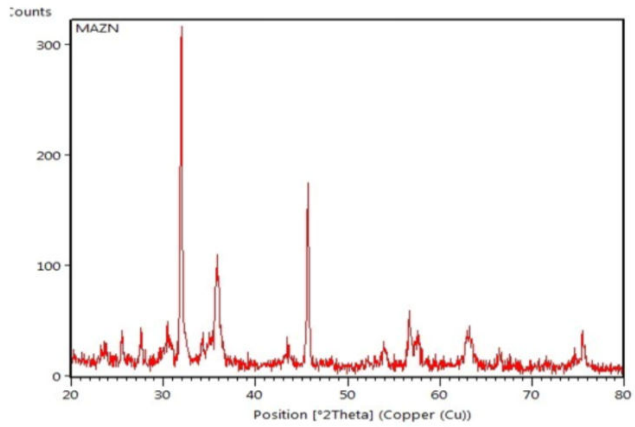

Figure 5. XRD analysis of synthesized IONPs.

AFM analysis: The confirmatory technique to characterize the synthesis of IONPs by AFM. The mean of their detecting average diameter were achieved from this study confirmed that the synthesized IONPs had average size of 25 nm with surface roughness 1.27 nm. The result agreed with the X-ray and TEM output. Figure 6 showed the topography surface, size, structure and height of Fe_2O_3 NPs which surveyed by AFM analyses.

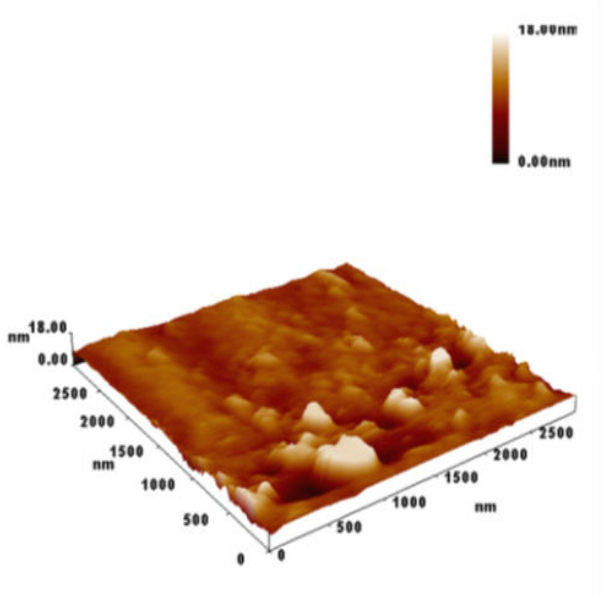

Figure 6. The synthesized IONPs 3 dimension AFM for IONPs.

TEM analysis: The morphology characterization was also carried out by the Transmission Electron Microscopy (TEM). Figure 7 represents the TEM image of IONs. These images confirm that ION is a scale of nanoparticles which the average size is in the range 25-30, grown in spherical shape, which demonstrates the good quality of the IONPs and have good homogeneity. This is in close agreement with the results obtained from powder XRD measurement.

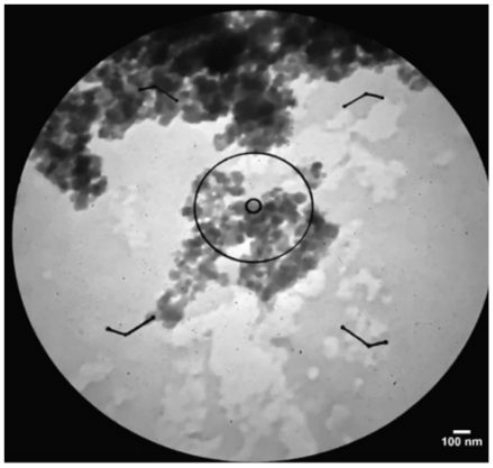

Figure 7. The Transmission Electron Microscopic (TEM) image of the IONPs.

Energy Dispersive of X-Ray spectrometry (EDX): Figure 8 shown EDX (Energy dispersive of X-Ray spectrometry) analysis for the nanoparticles which emphasis that the product is Fe_2O_3.

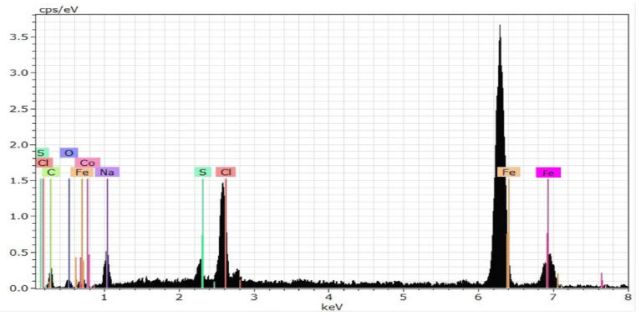

Figure 8. Energy dispersive of X-Ray spectrometry (EDX).

Zeta potential: The procedure for measuring the electrostatic potential at the electrical double layer around a nanoparticle in solution is described in this test. This is referred to as the zeta potential. Nanoparticles with a zeta potential between -10 and +10 mv are considered approximately neutral, while nanoparticles with zeta potential of greater than +30 mv or less than -30 mv are considered strongly cationic and strongly anionic, respectively since cellular membranes are negatively charged,zeta potential can affect a nanoparticles tendency to permeate membrane, with cationic particle generally displaying more toxicity associated with cell wall disruption. IONPs Nanoparticles with a zeta potential test has -19.54 as shown in Figure 9.

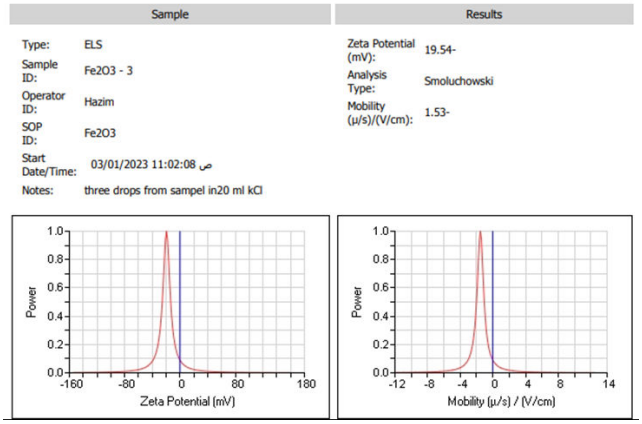

Figure 9. Showed the zeta potential of IONPs nanoparticles.

Field Emission Scanning Electron Microscopy Study (FESEM): Results clearly exhibited that the majority of the synthesized nanoparticles were spherical in shape. The average size of chemically synthesized IONPs was 19 nm-30 nm. Nanoparticles synthesized Ultrasound route were agglomerated in many areas which might be due to high surface energy [14].The sizes agglomeration of the IONPs and size of NPs are showed in Figure 10 for the SEM images for different magnifications (Figure 10).

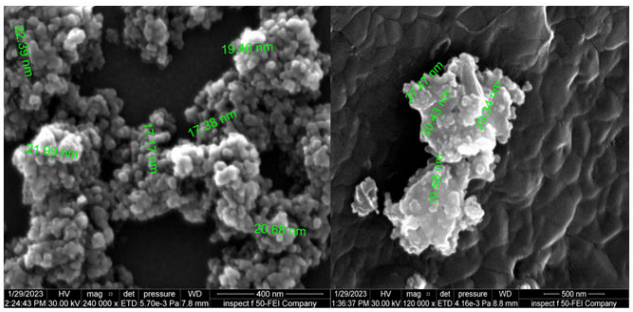

Figure 10. SEM images and size of IONPs.

Antibacterial activities: The antibacterial capacity of novel preparation IONPs by sonicater physical method was evaluated firstly against *S. aureus* by well diffusion method. The plates were incubated in (37°C), the inhibition zone diameter was evaluated which proof the antibacterial capacity. This experiment demonstrated that a higher concentration was (64 µg/ml) and the highest IZD value. In this case, a basic concentration of 16 µg/ml was employed to measure the MIC assay (Table 1 and Figure 11).

Different concentrations of IONPs diameter of inhibition zone (mm)					
Bacterial strains	**64 µg/ml**	**32 µg/ml**	**16 µg/ml**	**8 µg/ml**	**4 µg/ml**
S. aureus	15	13	10	8	5
Streptococcus pyogenes	16	14	11	7	4
Klebsiella	13	11	9	6	3
P. aeruginosa	16	14	10	7	4

Table 1. Results for IONPs effects on bacterial strain.

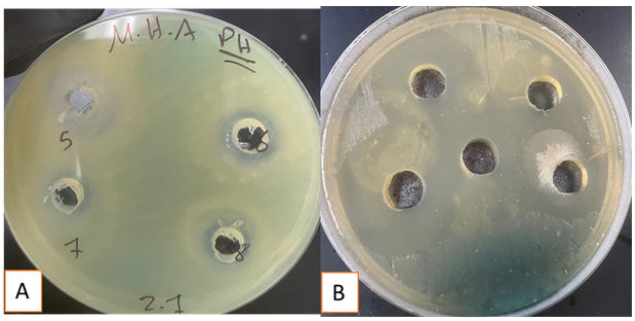

Figure 11. The inhibition zone values of IONPs different concentrations (64, 32, 16, 8,4) µg/ml against two bacteria strains A) *P. aeruginosa*; B) *Streptococcus pyogenes*.

The MIC values for IONPs against MDR was between 64 and 4 g/ml. *S. pyogenes* and *P. aeruginosa* had the highest result (64-4) µg/ml. In contrast, *Klebsiella* spp result revelated as that lowest amount of inhibition (4 µg/ml). Therefore from these result that *P. aeruginosa* and *Klebsiella*, are more resistant to antibiotics than *S. pyogenes*, which is a G^{ve+} bacteria. In G^{ve-} bacteria, mechanism of efflux pumps by multidrug as membrane-resistance [15,16].

The concentration range between (100, 200, 300, 400, 500) µg/ml of IONPs by Ultrasound assay resulted that the reduction in the number of UBC-40 cells. The hazardous oxygen species of photocatalytic activity were linked to the nano-iron. A reduction in cell viability causes cell death, such as cell membrane damage, and the toxicity is frequently linked to apoptosis. HdFn. The result investigated the significance of IONPs-induced toxicity in terms of the IC_{50} values for HdFn cells (Figure 12 and Tables 2,3).

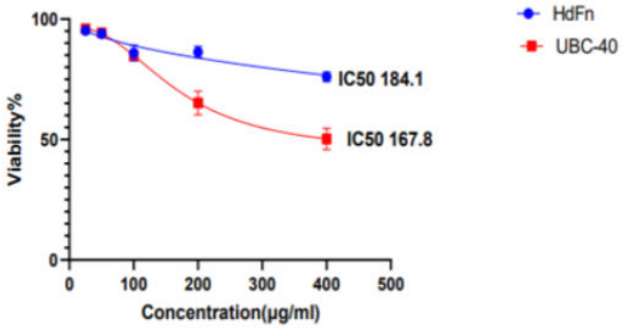

Figure 12. The cytotoxic effect of on HdFn and UBC-40 cell line.

(Inhibitor) *vs.* response variable slope (four parameters)	HdFn	UBC-40
Best-fit values		
Bottom	-8469	44.36
Top	101.6	96.73
IC_{50}	Unstable	167.8
Hill slope	-0.4927	-2.377
$logIC_{50}$	Unstable	2.225
Span	8570	52.38
95% CI (profile likelihood)		
Bottom	-	16.01-51.79
Top	-	93.19-102.6
IC_{50}	Very wide	138.1-283.8
HillSlope	-	-3.595-1.264
$logIC_{50}$	Very wide	2.140-2.453
Goodness of fit		
Degrees of freedom	11	11
R squared	0.8787	0.9802
Sum of squares	90.8	95.16
Sy.x	2.873	2.941
Constraints		
IC_{50}	IC_{50}>0	IC_{50}>0
Number of points		
X values	15	15
Y values analyzed	15	15

Table 2. The cytotoxic effect of on HdFn and UBC-40 cell line.

Concentration µg mL^{-1}	Mean viability (%) ± SD	
	HdFn	UBC-40
400	76.04 ± 1.9	50.23 ± 4.42
200	86.26 ± 2.38	65.239 ± 4.94
100	85.957 ± 3.12	84.72 ± 1.33
50	93.94 ± 0.06	94.29 ± 0.82
25	95.216 ± 0.65	95.949 ± 0.9

Table 3. The concentration and the mean viability.

Conclusion

The synthesis of IO NP by ultrasound assisted method which is used in this research is suitable to prepare IO NPs according to the size of particle and homogeny of the product. An alarming situation has been by multi resistant bacterial strains. Fe_2O_3 NPs were successfully synthesized following an in, direct, eco-friendly, low cost, high yield and green method, Iron nanoparticles showed exceptional antimicrobial activity against several bacterial strains.

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