

# Evaluation of antibacterial activity of Fe<sub>2</sub>O<sub>3</sub> nanoparticles against *Shigella dysenteriae*

Zainab H. Kareem<sup>1</sup> Hasanain Khaleel Shareef<sup>2</sup> Ayad F. Alkaim<sup>2\*</sup>

<sup>1</sup>College of science- Babylon University

<sup>2</sup>College of science for women- Babylon University

## Abstract

Fe<sub>2</sub>O<sub>3</sub> nanoparticles were synthesized using Hydrothermal method, Ag<sup>+</sup> ions on the Fe<sub>2</sub>O<sub>3</sub> nanoparticles reduced to the metal form using a photodeposition method. Thus, Ag/Fe<sub>3</sub>O<sub>4</sub> composite core-shell nanoparticles were synthesized. The products were characterized by Scanning electron microscope (SEM) and x-ray diffraction (XRD). Both SEM and XRD results showed that the Ag nanoparticles were well distributed on the surface of Fe<sub>2</sub>O<sub>3</sub> nanoparticles. The size for Ag/Fe<sub>3</sub>O<sub>4</sub> nanoparticles which were tubular shape was approximately 17 nm. Furthermore, Antibacterial activity for both Fe<sub>2</sub>O<sub>3</sub> and Ag/Fe<sub>2</sub>O<sub>3</sub> have been evaluated against gram negative bacteria *Shigella dysenteriae*.

**Keywords:** *Shigella dysenteriae*; antibacterial activity; nanoparticles

## 1- INTRODUCTION

The outbreak of infectious diseases caused by different pathogenic bacteria and the development of new resistant strains of bacteria to current antibiotics has become a serious problem in public health; therefore, there is a strong incentive to develop new antimicrobial agents [1].

Nanotechnology today provides a sound platform for adjusting the physicochemical properties of numerous materials to generate effective antimicrobials. Nanomaterials (NM), may be strategically advantageous as active antibacterial groups since their surface area is exceedingly large relative to their size. Nano sized particles may provide high activity although only a small dose of the particles is used. Consequently, Nanomaterials could serve as an alternative to antibiotics to control bacterial infections [2].

Metal oxides such as ZnO, TiO<sub>2</sub>, Fe<sub>2</sub>O<sub>3</sub> and CuO nanoparticles have been demonstrated as potential excellent antibacterial materials. In particular, Fe<sub>2</sub>O<sub>3</sub> nanoparticles, with a band gap of ~2.2 eV, have received increasing attention for bacterial inhibition in recent years due to its visible light absorption properties (~564 nm), unique magnetic properties and biocompatibility [3].

Release of iron oxide nanoparticle into the environment interact with air, water and soil often causes change in the surface properties of the particles which can result in particle aggregation or changes in particle charge and other surface properties. Various surface modifications are being done for making these non-biodegradable nanoparticles more biocompatible [4].

Surface modification is a method to improve the surface characteristics of iron oxide nanoparticles. Surface modification is used to prevent aggregation, enhances the compatibility of iron oxide nanoparticles with biological environment and also improves the stability in suspensions [5].

Silver nanoparticles plays a vital role in the development of new antimicrobial substances against a number of pathogenic microorganisms. These nanoparticles due to their smaller size could be very effective as they can improve the antibacterial activity through lysis of bacterial cell wall [6].

To best of my Knowledge few articles have been reported on the synthesis of Ag/Fe<sub>2</sub>O<sub>3</sub> nanoparticles. [7] have shown synthesis of various heteromers of Ag/Fe<sub>2</sub>O<sub>3</sub> nanoparticles and their bactericidal activity, [8] have synthesized Ag/Fe<sub>2</sub>O<sub>3</sub> nanoparticles using glucose. Recently [9] have synthesized Ag/ Fe<sub>2</sub>O<sub>3</sub> nanoparticles by employing green *Azadirachta indica* leaf extract assisted process for antibacterial, antifungal and anticancer properties.

Many researchers have reported the enhanced antimicrobial activity of Ag/Fe<sub>2</sub>O<sub>3</sub> nanoparticles as compared to Fe<sub>2</sub>O<sub>3</sub> nanoparticles alone [7, 9].

Many researchers suggest that the release of Ag<sup>+</sup> ions from Ag/Fe<sub>2</sub>O<sub>3</sub> nanoparticles play an important role in the antibacterial activity [7].

Ag/Fe<sub>2</sub>O<sub>3</sub> nanoparticles are very effective than Ag NP's alone in dealing with pathogens like bacteria, fungi which are multi drug resistant pathogens due to the controlled release of Ag<sup>+</sup> ions [9].

In this work, Fe<sub>2</sub>O<sub>3</sub> nanoparticles were synthesized by Hydrothermal method, furthermore Ag doping Fe<sub>2</sub>O<sub>3</sub> has been prepared by using a photodeposition method[10]. Both of the structural characterization and antibacterial activity of Fe<sub>2</sub>O<sub>3</sub> and Ag/Fe<sub>2</sub>O<sub>3</sub> nanoparticles against *shigella dysenteriae* bacteria were investigated.

## 2. MATERIALS AND METHODS

### 2.1. Synthesis of Fe<sub>2</sub>O<sub>3</sub>/Ag Composite

Two gram of Sodium Hydroxide (NaOH) was dissolved in 200 ml distilled water with constant stirring, then 1gm of FeCl<sub>3</sub>.6H<sub>2</sub>O was added to the solution and (...)by N<sub>2</sub>(Nitrogen gas) for 30 min with continuous stirring for 1-2 hrs to get a homogeneous solution followed by hydrothermal treatment in Teflon- lined stainless steel vessels for 24hrs in an oven that was preheated to 170°C. After the hydrothermal reaction, the products were cooled down to room temperature and the solid products inside the Teflon vessels were separated from the solution and washed several times with distilled water with ultrasonic device to remove any soluble salts until the pH of the wash became neutral(pH =7), the solid products were finally dried at 70 °C for 24 h in a dust-proof environment prior to photodeposition of Ag on it.

Ag/Fe<sub>2</sub>O<sub>3</sub> nanocomposite was prepared by photo deposition of Ag on the surface of Fe<sub>2</sub>O<sub>3</sub>, 2gm from Fe<sub>2</sub>O<sub>3</sub> nanoparticle and a proper amount (0.5%) of silver nitrate (AgNO<sub>3</sub>) were contained in a quartz cell, the distilled water and methanol with ratio 10 % v/v (H<sub>2</sub>O/ methanol) were added and purgulated by N<sub>2</sub>(Nitrogen gas) for 30 minute, then the system was irradiated with UVA light (wavelength 365nm and light intensity of 2.3 mW.cm<sup>-2</sup>) under continuous magnetic stirring for 10-12 hrs [10]. after irradiation, The obtained powder was separated and then the product was finally dried for 24h at 70°C.

### 2.2. Characterization

Ag/Fe<sub>2</sub>O<sub>3</sub> composite was characterized using X-ray powder diffractometer (XRD) and scanning electron microscope (SEM). The XRD used was Philips PW1710 BASED X-Ray Diffractometer with 2θ values range from 10° to 89.98° and Cu Kα radiation source (λ=1.54060) operating at 40 kV and 25 mA. The SEM used was Hitachi S-4800.

### 2.3. Antibacterial Activity of Fe<sub>2</sub>O<sub>3</sub>/Ag Composite

Antibacterial activities of the synthesized Fe<sub>2</sub>O<sub>3</sub> and Ag/Fe<sub>2</sub>O<sub>3</sub> nanoparticles were performed by agar well diffusion method. Pure culture *Shigella dysenteriae* were sub cultured in sterile nutrient

broth for 24 hrs at 37°C. After 24 hrs, the inoculum was spread with sterile cotton swab on Mueller Hinton agar (MHA) plates. Wells of 6 mm diameter were made using sterile cork borer and different concentrations (20, 40, 60, 80 and 100 µg/ml in sterile distilled water) of Fe<sub>2</sub>O<sub>3</sub> and Ag/Fe<sub>2</sub>O<sub>3</sub> nanoparticles were added into the respectively labeled well. The plates were left at 37°C for 24 hrs. and results were recorded by measuring the diameter of inhibition zone (mm) [11].

**3.RESULTS AND DISCUSSIONS**

The morphology of the synthesized Ag/Fe<sub>2</sub>O<sub>3</sub> composite was investigated by Scanning Electron Microscopy (SEM). It has been observed that the synthesized nanoparticles possess a nanotube shape. (Figure 1) and (Figure 2 )show low- and high-magnification SEM images of the Ag/Fe<sub>2</sub>O<sub>3</sub> nanotubes. The diameter of the poly-dispersed α-Fe<sub>2</sub>O<sub>3</sub> nanoparticles has been calculated to be in the range of 20-30nm nm and the average diameter of NPs is approximately 17 nm. The facets on the surface of the structures are clearly discriminable and appear very smooth.

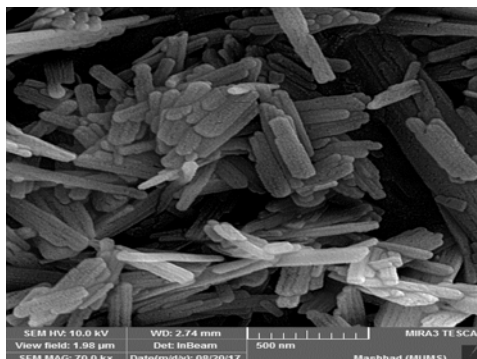


Figure 2. SEM of Ag/Fe<sub>2</sub>O<sub>3</sub> nanoparticles



Figure 1. SEM image of Fe<sub>2</sub>O<sub>3</sub> nanoparticles

**2.2. Antibacterial activity of Fe<sub>2</sub>O<sub>3</sub>/Ag composite**

The antibacterial activity of Fe<sub>2</sub>O<sub>3</sub> and Ag/Fe<sub>2</sub>O<sub>3</sub> composite against *S. dysenteriae* have been evaluated and zones of inhibition have been observed (Fig. 3 a and b). An increase in diameter of zone of inhibition could be observed on enhancing the concentration of the nanoparticles (Table 1), (Fig.4).

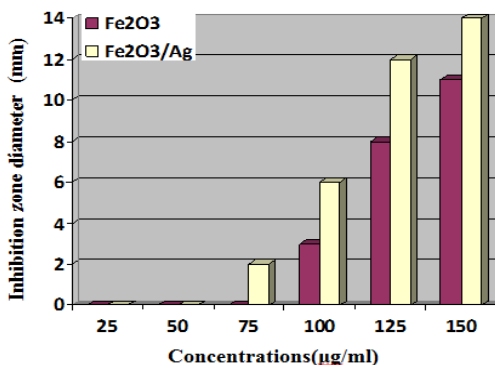


Fig.4. Antibacterial activity of Fe<sub>2</sub>O<sub>3</sub> & Ag/Fe<sub>2</sub>O<sub>3</sub> nanoparticles against *S. dysenteriae*.



Fig.3. Antibacterial test of a (Fe<sub>2</sub>O<sub>3</sub>) and b (Ag/Fe<sub>2</sub>O<sub>3</sub>) against *S. dysenteriae* in agar medium.

Table 1: Antibacterial activities of Fe<sub>2</sub>O<sub>3</sub> & Ag/Fe<sub>2</sub>O<sub>3</sub> nanoparticles against *S. dysenteriae*.

Type of nanoparticles	Zone of inhibition (mm)					
	150µg/ml	125µg/ml	100µg/ml	75µg/ml	50µg/ml	25µg/ml
Fe <sub>2</sub> O <sub>3</sub>	11	8	3	0	0	0
Fe <sub>2</sub> O <sub>3</sub> /Ag	14	12	6	2	0	0

The results showed that both Fe<sub>2</sub>O<sub>3</sub> and Ag/Fe<sub>2</sub>O<sub>3</sub> have antibacterial effects, antibacterial activity of Ag/Fe<sub>2</sub>O<sub>3</sub> nanoparticles was much higher than that of Fe<sub>2</sub>O<sub>3</sub> nanoparticles alone.

This results in a more efficient antibacterial activity is due to the presence of silver at the surface of Fe<sub>2</sub>O<sub>3</sub> nanoparticles, thus giving a higher antibacterial efficacy against *S. dysenteriae* bacteria.

Gram-negative bacteria exhibit only a thin peptidoglycan layer (ca. 2–3 nm) between the cytoplasmic membrane and the outer membrane. We believe that when the silver-doped Fe<sub>2</sub>O<sub>3</sub> nanoparticles were dispersed in the growth media, the silver atoms present in these particles interacted with the bacterial cells and adhered to the bacterial cell walls.

The overall charge on the bacterial cell surface at biological pH values is negative, which is due to the excess number of carboxylic and other groups that upon dissociation make the cell surface negative [12]. The bacteria and the silver atoms in the nanoparticles have opposite charges, and these electrostatic forces may be the reason for their adhesion and bioactivity.

It has been reported that ionic silver strongly interacts with thiol group (-SH) of vital enzymes and inactivates the enzyme activity, experimental evidence indicates that DNA loses its replication ability once the bacteria have been treated with silver ions [13].

Ag<sup>+</sup> ions inactivate the enzymes present in the bacterial cell by generating reactive oxygen species (ROS) like superoxide radical, hydroxyl radical and the hydrogen peroxide causing the death of the bacterial cell [14-18].

#### CONCLUSION

Both Fe<sub>2</sub>O<sub>3</sub> and Ag/Fe<sub>2</sub>O<sub>3</sub> have antibacterial effects, antibacterial activity of Ag/Fe<sub>2</sub>O<sub>3</sub> nanoparticles against *S. dysenteriae* was much higher than that of Fe<sub>2</sub>O<sub>3</sub> nanoparticles alone.

#### REFERENCES:

- Mishra, V., R. Sharma, N. Jasuja, and D. Gupta, *A review on green synthesis of nanoparticles and evaluation of antimicrobial activity*. Int J Green Herbal Chem, 2014. **3**: p. 081-094.
- Sharma, M., *APPLICATIONS OF NANOPARTICLES AS ANTIBACTERIAL AND ANTIMICROBIAL AGENTS WITH REFERENCE TO SILVER, GOLD AND ZINC METALS*. 2017.
- Long, M., Y. Zhang, Z. Shu, A. Tang, J. Ouyang, and H. Yang, *Fe<sub>2</sub>O<sub>3</sub> nanoparticles anchored on 2D kaolinite with enhanced antibacterial activity*. Chemical Communications, 2017.
- Elsaesser, A. and C.V. Howard, *Toxicology of nanoparticles*. Advanced drug delivery reviews, 2012. **64**(2): p. 129-137.
- Arakha, M., et al., *Antimicrobial activity of iron oxide nanoparticle upon modulation of nanoparticle-bacteria interface*. 2015. **5**: p. 14813.
- Patra, J.K. and K.-H. Baek, *Antibacterial activity and synergistic antibacterial potential of biosynthesized silver nanoparticles against foodborne pathogenic bacteria along with its anticandidal and antioxidant effects*. Frontiers in microbiology, 2017. **8**.
- Chen, Y., N. Gao, and J. Jiang, *Surface Matters: Enhanced Bactericidal Property of Core-Shell Ag-Fe<sub>2</sub>O<sub>3</sub> Nanostructures to Their Heteromer Counterparts from One-Pot Synthesis*. Small, 2013. **9**(19): p. 3242-3246.
- Kaloti, M., A. Kumar, and N.K. Navani, *Synthesis of glucose-mediated Ag-γ-Fe<sub>2</sub>O<sub>3</sub> multifunctional nanocomposites in aqueous medium—a kinetic analysis of their catalytic activity for 4-nitrophenol reduction*. Green Chemistry, 2015. **17**(10): p. 4786-4799.
- Kulkarni, S., M. Jadhav, P. Raikar, D.A. Barretto, S.K. Vootla, and U.S. Raikar, *Green synthesized multifunctional Ag@Fe<sub>2</sub>O<sub>3</sub> nanocomposites for effective antibacterial, antifungal and anticancer properties*. New Journal of Chemistry, 2017. **41**(17): p. 9513-9520.
- Alkaim, A.F., E.M. Alrobayi, A.M. Algubili, and A.M. Aljeboree, *Synthesis, characterization, and photocatalytic activity of sonochemical/hydration-dehydration prepared ZnO rod-like architecture nano/microstructures assisted by a biotemplate*. Environmental technology, 2017. **38**(17): p. 2119-2129.
- Ravikumar, S., R. Gokulakrishnan, K. Selvanathan, and S. Selvam, *Antibacterial activity of metal oxide nanoparticles against ophthalmic pathogens*. International Journal of Pharmaceutical Research and Development, 2011. **3**(5): p. 122-127.
- Stoimenov, P.K., R.L. Klinger, G.L. Marchin, and K.J. Klabunde, *Metal oxide nanoparticles as bactericidal agents*. Langmuir, 2002. **18**(17): p. 6679-6686.
- Joshi, D.D., *Herbal drugs and fingerprints: Evidence based herbal drugs*. 2012: Springer Science & Business Media.
- Xia, T., et al., *Comparison of the abilities of ambient and manufactured nanoparticles to induce cellular toxicity according to an oxidative stress paradigm*. Nano letters, 2006. **6**(8): p. 1794-1807.
- Al-Gubury, H.Y., Hassan, A.F., Alteemi, H.S., Alqaragully, M.B., Benneker, A., Alkaim, A.F., *Photo catalytic removal of paraquat dichloride herbicide in aqueous solutions by using TiO<sub>2</sub> nanoparticle*, 16. Journal of Global Pharma Technology, 12(9), 2017, 290-295.
- Kareem, A. Alrazak, N.A., Aljebori, K.H., Aljeboree, A.M., Alboory, H.L., Alkaim, A.F. *Removal of methylene blue dye from aqueous solutions by using activated carbon/ureaformaldehyde composite resin as an adsorbent*, International Journal of Chemical Sciences, Volume 14, Issue 2, 2016, 635-648.
- Abd Alhussain, A.J., Abd, F.G., Alkaim, A.F. *Biological synthesis and characterization of silver nanoparticles using Bacillus subtilis*, Journal of Global Pharma Technology Volume 9, Issue 9PartB, 2017, 239-244.
- Abd, F.G., Al-Shalah, L.A.M., Alkaim, A.F. *Effect of silver nanoparticles that extracted by staphylococcus aureus on cellular immunity in rabbits*, Journal of Pharmaceutical Sciences and Research Volume 10, Issue 6, June 2018, 1623-1624