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Antibacterial activity of Fe_3O_4 nanoparticles

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ABSTRACT: Antibacterial activity of Fe_3O_4 nanoparticles was investigated in three microorganisms including P. aeruginosa, E. Coli and Staphylococcus aureus. Fe_3O_4 nanoparticles were synthesized by chemical precipitation method (20 nm). The Fe_3O_4 nanoparticles antibacterial effect against Pseudomonas aeruginosa, Escherichia coli and Staphylococcus aureus were studied by the culture method. First of all, each bacterium was grown in nutrient broth culture environment aerobically for 24 h at 37°C. After that, about 1 mL of each bacterium was placed onto a plate which was previously prepared by Mueller Hinton Agar culture environment. Their characteristics and physical properties were evaluated by X-ray diffraction (XRD), Alternating Gradiant Force Magnetometer (AGFM) and Transmission Electron Microscope (TEM). Antibacterial effects of Fe_3O_4 nanoparticles (1.56 to 25 mg/mL concentrations) against three pathogenic bacteria including Pseudomonas aeroginosa, Escherichia coli and Staphylococcus aureus were studied using well diffusion bacteriological test. The inhibition zone diameter was directly and strongly correlated to the nanoparticles concentration and Pseudomonas aeroginosa was the most sensitive bacterium to the Fe_3O_4 nanoparticles. The maximum diameter of inhibition zone was occurred for P. aeroginosa bacteria for both Fe_3O_4 samples. The most sensitive bacterium among three pathogenic bacteria tested was P. aeroginosa.

Keywords: *Antibacterial effect; Escherichia coli; Fe_3O_4 nanoparticles; Pseudomonas aeroginosa; Staphylococcus aureus*

INTRODUCTION

Nanoparticles are relatively new materials which have a more effective interaction with biological systems due to their high proportion of surface to volume ratio in comparison with bulk ones. This characteristic has made them appropriate to be used in many medical and biological applications. This fact also has made them as high risk materials and increases their toxicity risk for biological systems usage. On the other hand, toxicity could be useful in some cases. For instance, some bacteria may be affected by nanoparticles which may lead to decrease of their harmful effects.

Super paramagnetic property is a behavior which is only could be found in nano size particles. This property exists in particles which are less than a specific size (critical size). The critical size is different for every nanoparticle which could be a super paramagnetic material. Iron oxide nanoparticles are among those nanoparticles which exhibit the super paramagnetic behavior at 30 nm critical size and below. Super paramagnetic iron oxide nanoparticles (SPION) with ap-

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propriate surface chemistry can be used for numerous applications such as in vitro enhancing resolution in MRI, tissue repair, detoxification of biological fluids and hyperthermia (Amiri, *et al.*, 2010, Amiri, *et al.*, 2012).

There are many methods which can be used for synthesizing nanoparticles such as co-precipitation, low temperature solid state reaction and sol gel. One of the most commonly used methods for synthesizing SPION is wetted chemical method which is used in this study (Rizwan, *et al.*, 2010, Sobha, *et al.*, 2010, Amiri, *et al.*, 2014, Fashen, *et al.*, 2007, Amiri, *et al.*, 2013).

Nanoparticles have a great range of probable interaction with cell surface due to the large surface to volume ratio in comparison with bulk ones (Rizan, *et al.*, 2010). Some nanoparticles like ZnO, CdSe, TiO_2, ZnS and SiO_2 have considerable antibacterial activity and selective toxicity in biological systems which were previously reported (Sobha, *et al.*, 2010). In this study, an attempt was made in order to investigate the antibacterial effect of Fe_3O_4 nanoparticles on three pathogen bacteria including Pseudomonas aeroginosa, Escherichia coli, and Staphylococcus aureus).

MATERIALS AND METHODS

Synthesis and assessment of properties

Wetted chemical method was used in order to synthesize Fe_3O_4 nanoparticles (Amiri, *et al.*, 2013). For this purpose, three solutions of $FeCl_2$ (0.01 M), $FeCl_3$ (0.02 M) and NaOH (0.08 M) (Merck, Germany) were prepared in the distilled deionized water.

First, $FeCl_2$ solution was poured into a triple-neck round bottom flask and $FeCl_3$ solution in water was added to the same flask under vigorous stirring. Then, 50 µl mercaptoethanol (ME) was added. Then, the NaOH solution was added to the mixture and finally, the prepared solution was washed by deionized water. In order to remove any impurity aggregate, the solution was centrifuged. All of the mentioned processes were repeated when starch (S) was used instead of mercaptoethanol.

Antibacterial activity assay

The antibacterial effect of Fe_3O_4 nanoparticles against P. aeruginosa, E. coli and S. aureus were studied by culture method. First of all, each bacterium was grown in nutrient broth culture environment aerobically for 24 h at 37°C. After that, about 1 mL of each bacterium was placed onto a plate which was previously prepared by Mueller Hinton Agar culture medium. Then, a well was created in every plate by the Pasteur pipette. The concentrations of 1.56 to 25 mg/mL of both Fe_3O_4 (ME) and Fe_3O_4 (S) were adjusted in wells separately. After inoculation and culturing of different target bacteria on the top of nutrient agar, wells were made in selected areas on different plates. The diameter of inhibition zone was assessed 24 h post-incubation.

Measurement of properties

Both samples of synthesized Fe_3O_4 nanoparticles using either mercaptoethanol or starch were assessed by XRD (X-ray Diffraction, Bruker D8 ADVANCE λ= 0.154 nm Cu Kα radiation). The accelerating voltage and the applied current were 40 kV and 40 mA, respectively. Data were recorded at a scan rate for two seconds in steps of 0.04° for 2θ. The crystalline size was calculated from X-ray line broadening analysis by the Debye-Scherer equation for the full-width at half-maximum of the strongest reflection where, D is the crystalline size in nm, λ is the Cu-Kα wavelength (0.154 nm), β is the half-maximum breadth, and θ is the Bragg angle of the (311) plane (Fashen, *et al.*, 2007):

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

For more accuracy TEM (Mode: BF, HT: 150 kV) was prepared from the Fe_3O_4 (S) sample. Moreover, the AGFM (Alternating Gradient Force Magnetometer, Meghnatis Daghigh Kavir Co, Iran) was prepared from both samples in order to assess the magnetic properties.

RESULTS AND DISCUSSION

Physical properties investigation

XRD patterns of both Fe_3O_4 nanoparticle samples are shown in Figs. 1 and 2. It can be seen that both samples are single phase and have the ferrite spinel

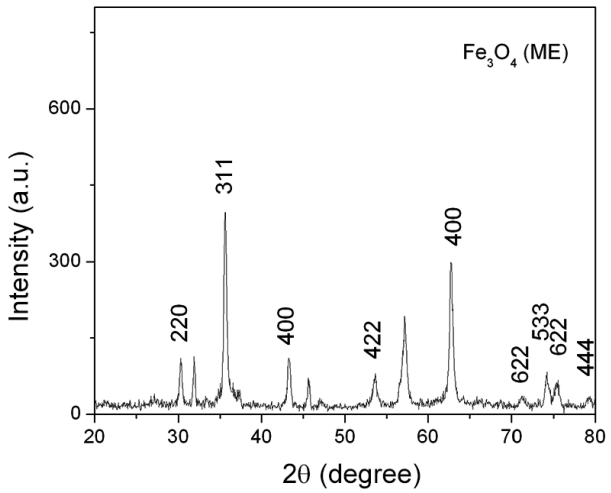


Fig. 1: XRD pattern of Fe_3O_4 samples produced by mercaptoethanol as inhibitor.

structure. The size of the samples can be calculated by Scherer equation which is 23 nm for Fe_3O_4 (S) and 22 nm for Fe_3O_4 (ME).

In order to measure the magnetic properties of ferrite nanostructure samples, magnetic hysteresis curve prepared using AGFM. Figs. 3 and 4 illustrate the hysteresis curve for both Fe_3O_4 (S) and Fe_3O_4 (ME) samples. As can be seen, both plots show the superparamagnetic property and the saturation magnetization (around 15 emu/gr) occurred at 4000 Oe.

Fig. 5 shows the TEM micrograph of the Fe_3O_4 samples produced by starch as inhibitor. It is clear that TEM is one of the most important and widely used methods for determining particles size and size distribution with an accuracy of less than a nanometer. The

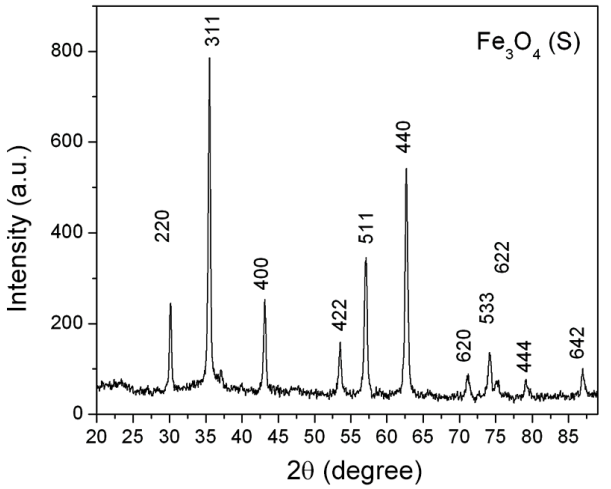


Fig. 2: XRD pattern of Fe_3O_4 samples produced by starch as inhibitor.

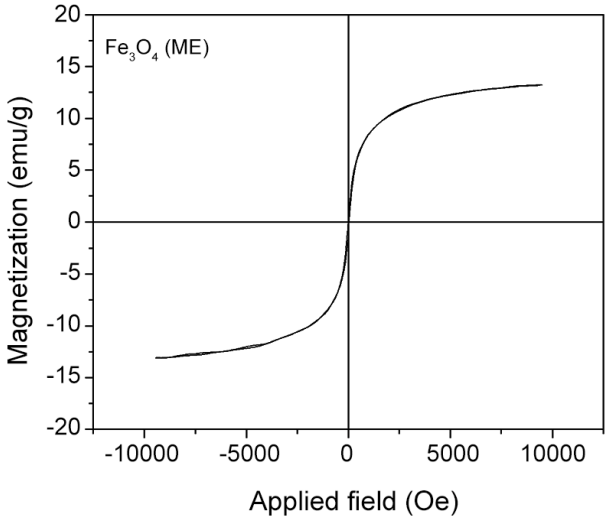


Fig. 3: Magnetic hysteresis curve of samples produced by mercaptoethanol inhibitor Fe_3O_4 (ME).

size of the Fe_3O_4 (S) nanoparticles is around 20 nm from the TEM photograph.

Antibacterial assessment

Well diffusion agar method was used in order to investigate the antibacterial activity of Fe_3O_4 nanoparticles. The results are shown in Tables 2, 3 and Fig. 6. The diameter of the inhibition zone shows the antibacterial effect activity of the Fe_3O_4 nanoparticles. It can be seen that, the inhibition zone has increased due to increase the Fe_3O_4 concentration in wells. Moreover, the diameter of the inhibition zone was completely different according to the different type of bacteria.

According to the results obtained from Tables 2 and

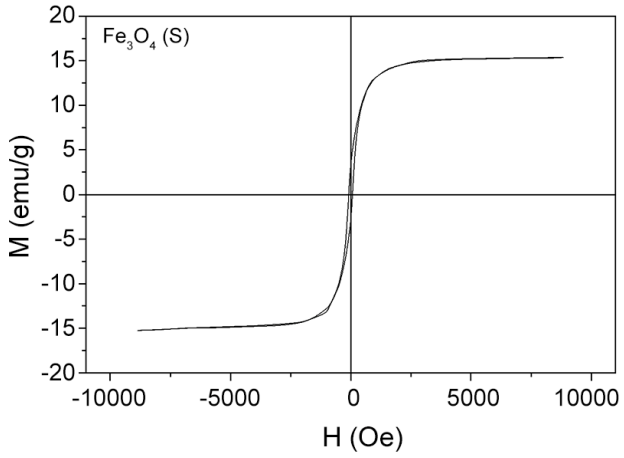


Fig. 4: Magnetic hysteresis curve of Fe_3O_4 samples produced by starch as inhibitor.

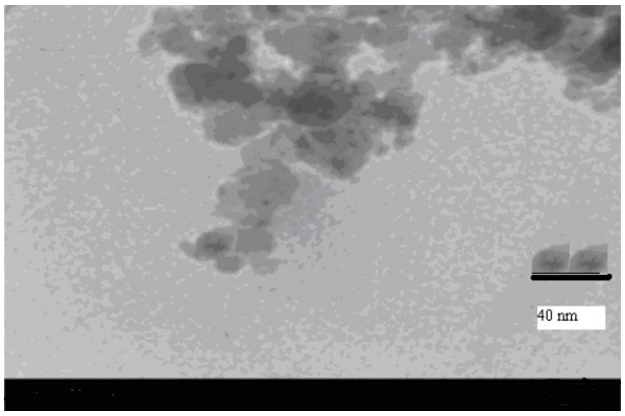


Fig. 5: TEM micrograph of Fe_3O_4 samples produced by starch as inhibitor.

3 the maximum inhibition activity is happened for P. aeroginosa bacteria in 25 mg/mL concentrations. Fig. 6 demonstrated the similar results which are completely agreed with Tables 2 and 3.

As it was also shown in the study of (Senthil *et al.*, 2012) it has been seen in this study that by increasing the concentration of Fe_3O_4 nanoparticles in wells and discs, the growth inhibition has also been increased. The size of inhibition zone was different according to the type of bacteria, the size and the concentrations of Fe_3O_4 nanoparticles (Reddy *et al.*, 2007) have reported the same results, emphasizing on the higher susceptibility of Gram-positive bacteria in comparison with Gram-negative bacteria. In the study done by (Selahattin *et al.*,1998), it has been proposed that the higher susceptibility of Gram-positive bacteria could be related to differences in cell wall structure, cell physiology, metabolism or degree of contact.

Table 1: Structural and magnetic properties of the synthesized nanoparticles.

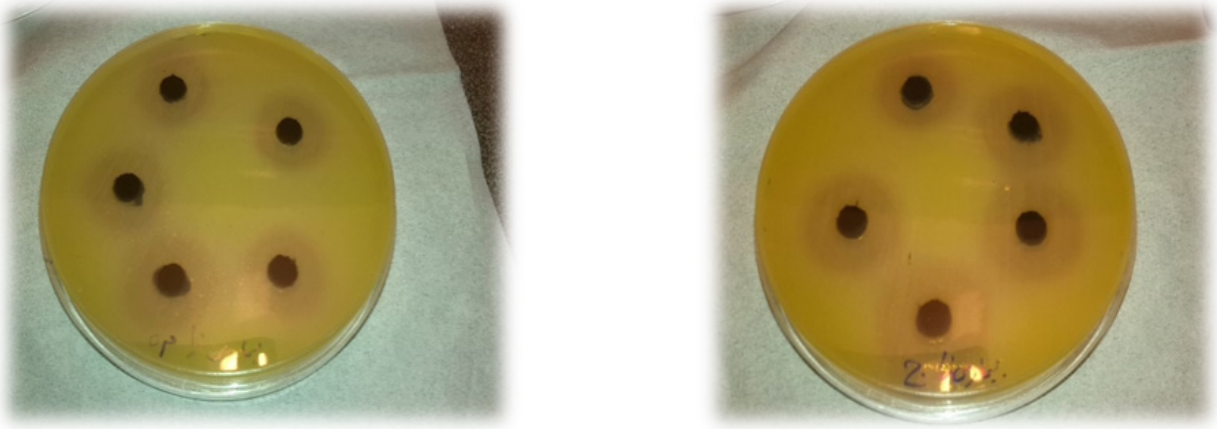
Specimen	Size by XRD	Size by TEM	Hc (Oe)	Mr (emu/g)	M (emu/g)
Fe_3O_4(ME)	22	-	20	0.5	13.2
Fe_3O_4(S)	23	20	80	3.25	15

Table 2: The diameter of inhibition zone for Fe_3O_4 samples produced by mercaptoethanol as inhibitor.

Concentration (mg/mL)	P. aeroginosa (mm)	E. coli (mm)	Staphylococcus aureus (mm)
25	24	6	5
12.5	7	5	5
6.25	5	5	5
3.125	5	5	5
1.56	5	5	5

Table 3: The diameter of inhibition zone for Fe_3O_4 samples produced by starch as inhibitor.

Concentration (mg/mL)	P. aeroginosa (mm)	E. coli (mm)	Staphylococcus aureus (mm)
25	23	5	5
12.5	7	5	5
6.25	5	5	5
3.125	5	5	5
1.56	5	5	5



Fe_3O_4 (ME) Fe_3O_4 (S)

Fig. 6: Antibacterial activity of both Fe_3O_4 samples against P. aeroginosa (25 mg/mL).

CONCLUSIONS

In this research, Fe_3O_4 nanoparticles were produced by wetted chemical method (20 nm) using ME and starch as an inhibitor separately. It can be concluded that the size of Fe_3O_4 nanoparticles can be controlled by the type of inhibitor and inhibitor concentration. It is also concluded that the size of the nanoparticles can affect on their antibacterial properties and the diameter of inhibition zone.

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REFERENCES

Amiri G.R.; Yousefi M. H.; Aboulhassani M. R.; Keshavarz M. H.; Shahbazi D.; Fatahian S.; Alahi M.; (2010). Badr absorption of Ni 0.7, Zn 0.3 Fe2O4 nanoparticles, Dig J Nanomater Biostruct., 5(3): 1025.

Amiri G.R.; Yousefi M.H.; Fatahian S.; (2012). Preparatin and investigation of Ni1-xZnxFe_2O_4 nanoparticles: the effect of x parameter on the magnetic properties, Optoelectron Adv, Mat., 6(5-6): 564-567.

Rizwan W.; Amrita M.; Soon-Il Y.; Young-Soon K.; Hyung-Shik Sh.; (2010). Antibacterial activity of ZnO nanoparticles prepared via non-hydrolytic solution route, J. Appl. Microbial. Biotechnol., 87(5): 1917-1925.

Sobha K.; Surendranath K.; Meena V.; Jwala K.T.; Swetha N.; Latha K.S.M.; (2010). Emerging trends in nanobiotechnology. J. Biotech. Mol. Bio. Rev., 5(1): 001-012.

Amiri G.R.; Fatahian S.; Kianpour N.; (2014) Investigation of ZnS nanoparticle antibacterial effect, Curr. Nanosci., 10(6): 796-800.

Fashen W.H.; Wang L.; Wang J.; (2007). Magnetic properties and crystal structure of the hydrides of ferromagnetic, J. Magn. Magn. Mater., 295-309.

Amiri G.R.; Fatahian S.; Mahmoudi S.; (2013). Preparation and optical properties assessment of CdSe quantum dots, J. Mater. Sci. Appl., 4: 134-137.

Senthil M.; Ramesh C.; (2012). Biogenic synthesis of Fe3O4 nanoparticles using tridax procumbens leaf extract and its antibacterial activity on pseudomonas aeruginosa. Dig J Nanomater Biostruct., 3: 1655-1660.

Reddy K.M.; Kevin F.; Jason B.; Denise G.W.; Cory H.; Alex P.; (2007). Selective toxicity of zinc oxide nanoparticles to prokaryotic and eukaryotic systems, J. Appl. Phys. Lett., 90(21): 1-3.

Selahattin A.; Kadri Ramazan G.; (1998). The effect of zinc on microbial growth, Tr. J. Med. Sci., 28: 595-597.

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