RESEARCH ARTICLE

Synthesis and Biochemical effects of magnetite nanoparticle by surfactant-free electrochemical method in an aqueous system: The current density effect

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ABSTRACT

Obejective: In this research, magnetite nanoparticles with an average size of 23-36 nm were successfully synthesized via surfactant-free electrochemical method using iron as the anode and water as the electrolyte in a closed aqueous system in the presence of NaOH at room temperature.

Methods: The effect of the current density on product formation and particle size was investigated. Particle size was controlled by adjusting the current density. It was found that particle size decreases by decreasing the current density. In addition, the effect of current density on the structural and optical properties of nanostructures were studied by X-ray diffraction, Field emission scanning electron microscopy, Fourier transformed infrared, and vibrating sample magnetometer techniques.

Results: The results obtained from the magnetization property study of samples at room temperature showed coactivity and saturation manetization of 0-100 Oe and 27.2- 40.5 emu. g^{-1}, respectively. Finally, the results of biological activity study of nanoparticles on liver and kidney function in male wistar rats demonstrated that oral administration of NPs caused significant alterations to the levels of aspartate transaminase, alanine transaminase, and alkaline phosphatase in serum.

Conculosion: No significant changes were detected in the groups treated with 10 and 100 ppm/ day nanostructure ($P>0.05$). There was a significant increase in the serum level of creatinine and blood urea nitrogen level ($p<0.05$) in rats treated with high oral doses of the nanostructure.

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INTRODUCTION

In the recent years, study of the nano-sized magnetite particles of iron oxide has been increased due to their wide applications in hyperthermia treatment, targeted drug delivery, magnetic resonance imaging, cell separation, isolation of biochemical products, immunoassays, and treatment of water and wastewater because of strong superparamagnetic properties, low toxicity, and biocompatibility in the human body and simple synthesis methods [1-9].

During the past several years, the different methods have been employed to prepare the

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iron oxide nanoparticles such as sol-gel [10], hydrothermal [11], solid state [12], wet milling [13], pyrrolysis [14], and microemulsion [15]. In addition, the electrochemical method has been also used to synthesize the iron oxide nanoparticles in the absence or presence of surfactant [16–19]. The electrochemical method suggests many advantages over the other methods of iron oxide nanoparticle synthesis. Using this method, the particle size can be easily controlled by adjusting factors such as the current density and the distance between the electrodes.Synthesis of nano-sized particles of iron oxide using surfactant-free electrochemical method via liquid phase reactions has been reported in the literature [22]. Marques *et al.* produced magnetite nanoparticles in the size range of 4.4 - 9 nm using $Fe(NO_3)_3$ as the iron source in ethanol media by the electrochemical processes [16]. Franger *et al.* synthesized nanomagnetit in the size range of 74-88 nm by electrochemical method using an iron-based electrode in an alkaline aqueous solution [18, 19]. Cabrera *et al.* used a similar method to synthesize iron oxide nanoparticles in the size range of 20-30 nm in aqueous solution. In this study, pure iron was employed as the anode, and an amine surfactant helps to prevent aggregation of nanoparticles [17]. Due to the side effects of synthesizing iron oxide nanoparticles in the presence of surfactants in biomedical applications, several studies have been reported the surfactant-free electrochemical method for the magnetit nanoparticles synthesis using liquid phase reactions [20-22]. Nishio *et al.* prepared magnetite nanoparticles in the size range of 30-100 nm by oxidizing ferrous hydroxide in reaction with a weak oxidant in a N_2-deaerated aqueous NaOH solution at different temperatures and in the absence of surfactant [20]. Fauziatul *et al.* synthesized magnetite nanoparticles in the size range of 10-30 nm by surfactant-free electrochemical method using iron as the anode and plain water as the electrolyte [22].

In the current study, we synthesized the iron oxide nano particles using an electrolytic cell without separator page in the closed water system. The synthesis process took palce with no application of nitrogen or argon gas, in the absence of surfactant and capping agent, and changing the current density using an iron anode and water as the electrolyte in the. The characterization techniques such as scanning electron microscopy (SEM), and X-ray diffraction (XRD) were used to investigate the particle sizes, the morphology, and the structure of the prepared nanomagnetits. Biochemical effects of iron oxide nanoparticles on liver and kidney function in male wistar rats were also studied as part of the investigation.

EXPERIMENTAL

In order to prepare the magnetite nanoparticles, first, 4 gr of iron sulfate (II) ($FeSO_4$) with molar mass of 278.02 g $mole^{-1}$ was dissolved in 200 mL of the double distilled water. Then, two steel plates (13 × 23 mm) with a thickness of 0.5 mm were placed under electroplating in a 0.072 M $FeSO_4$ solution under the current density of 13.04 mA cm^{-2} for 3 hours [22].

In the first step of plating process to obtain a layer of highly pure iron, after preparation of solutions, ammeter connected in series in the circuit. During the experiment, the electric current was set on 15 mA using the Power Supply. Three hours after plating by replacing the anode and cathode, NaOH solution was added drop by drop to the system [22].

In this research, iron sticking to the steel and water played the role of anode and cathode, respectively. By keeping the distance between the electrodes, the current density was vvariable in magnetite nanoparticles synthesis [22].

The deposited materials were washed with double distilled water by centrifugation. The resulting material was dried for 2 hours at 60 ° C. Under the time course of the experiment, the certain mass value of dried powders was obtained depending on applied current density [22]. The characterization of the magnetite nanoparticles was done using the XRD, Fourier transformed infrared (FTIR), vibrating sample magnetometer (VSM), and Field emission scanning electron microscopy (FESEM) techniques. The experiments were carried out with deionized water at room temperature. The XRD patterns were detected using an X-ray diffractometer (D8 advance Bruker, X'Pert). The magnetic characterisattion of nanoparticles was investigated using VSM technique (Kavir Daghigh Magnetic Co, Iran). The FESEM image of the prepared sample was obtained by field emission spectroscopy electron microscope (Mira 3-XMU model) with great features and speed vacuum chamber Balaamkan image to zoom in 700,000 times, and the possibility of structural checking in the nano-meter scale.

Biological activity

In order to study Biological activity of prepared nanoparticles, adult male rats (190- 225 g) were

kept in plastic cages at 21–22 °C, humidity about 56%, and 12 h light/dark cycles with free access to water and pelleted food (Javaneh Khorasan Co, Mashhad, Iran). The animals were equally divided into four groups (n = 10). The control group received normal saline. The second group was treated with of magnetic nanopartcles (10 ppm) for two weeks through gastric tube. The third group received magnetic nanopartcles (100 ppm). The animals of forth group were treated by 1000 ppm of nanostructure. The blood samples of all rats were centrifuged in order to separate the serum, and immediately frozen at -80 °C. Then, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were determined using commercial kits. The obtained results were analyzed using SPSS software and expressed as mean ± standard deviation. Furthermore. multiple comparisons were performed by ANOVA using Dunnett tests. The critical differenceset (CD) was set at $P<0.05$.

RESULTS AND DISCUSSION

The iron oxide nanoparticles were synthesized by electrochemical method based on variation of current density in the presence of NaOH. The black color precipitate was identified as magnetite by XRD technique. Fig.1 shows the XRD patterns for three representative samples (1,2 and 3) with the addition of NaOH under the changing of the current density. In Table 1, specific parameters used in the electrochemical synthesis of iron oxide nanoparticles has been shown.

As shown in Fig. 1, The Bragg peaks (indicated by a circle symbole)were obtained at 2Θ values of 30.5° (2 2 0), 35.9° (3 1 1), 43.5° (4 0 0), 53.5° (4 2 2), 57.3° (5 1 1), 63.1° (4 4 0), and 74.1° (533) which showed goodagreement with the standard pattern of Fe_3O_4 (JCPDS 00-003-0863).

There are also diffraction peaks (indicated by the square symbol) which greatly correspond with the standard pattern of FeOOH (JCPDS 00-018-0639). The average crystallite size (D) of magnetic nanoparticles was calculated by the Scherer formula using (311) plane reflection of the XRD pattern [23-24]:

$$D_{h,k,l}=0.9\lambda/ (\beta_{h,k,l} \cos\theta) \quad (1)$$

Where λ is the wavelength (λ = 1.542 Å) (CuK_α),

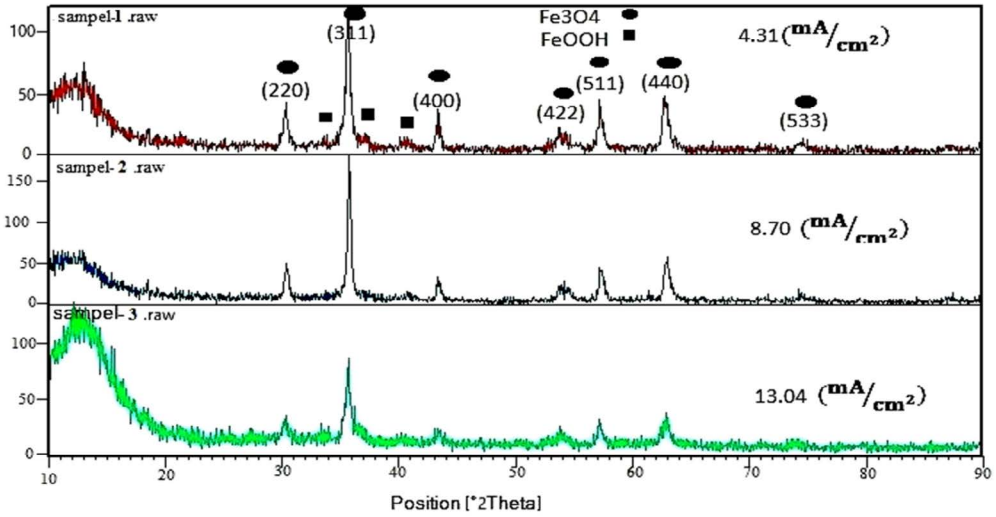

Fig.1. XRD patterns of the nanoparticles prepared by electrochemical method in presence of NaOH and by changing the current density. The distance between electrodes wes adjusted to be 4 cm.

Table 1. Results of the current density enhancement in the synthesis of magnetite nanoparticles in the closed water system

Samples	Distance electrods (cm)	pH	Water system case	Current density (mA cm^{-2})	Average crystallite size (nm)
1	4	13	closed	4.31	23
2	4	13	closed	8.70	26.4
3	4	13	closed	13.04	36

β is the full width at half maximum of the line, and θ is the diffraction angle (Table 2).

Fig. 2 shows FTIR spectra that were taken on JASCO 640 plus infrared spectrometer in the wavelength range of 4000–400 cm^{-1} at room tempreture.

Samples were prepared by mixing their powdered-form with KBr, which were then pressed and placed into a transparent pellet with a diameter of 2 cm.

The FTIR spectrum of the final product (Fig. 2) confirms the formation of Fe_3O_4 nanoparticles. The IR spectrum displays two distinct and sharp bands at 583.35 (ν_1) and 796.53 (ν_2) cm^{-1}, which originate from the stretching vibrations of the metal–oxygen bond and confirm the formation of Fe_3O_4 spinel oxide. The ν_1 band is related to Fe^{3+} vibration in the octahedral hole, and ν_2 band is attributed to Fe^{2+} vibration in a tetrahedral hole in the spinel lattice [27-29]. The intensive broadband at 3435.51 cm^{-1} and 1636.63 are due to O–H stretching vibration interacting through hydrogen bonds [29]. The absorption bond at 1127.93 cm^{-1} associated to the H_2O molecules. The absorption bonds at 888.78 and 977.12 cm^{-1} corresponds with bending vibration related to the out of plane bonds of O-H [27].

The magnetic properties of the synthesized magnetite nanoparticles were studied with the VSM technique at room temperature. Fig. 3 shows the magnetization curve for magnetite nanoparticles prepared at a distance of 3 cm between electrodes with three different current densities (4.31, 8.70, and13.0 4 mA cm^{-2}). The VSM result indicates that the magnetic nanoparticles likely possess ferromagnetic properties as the achieved hysteresis loopis typically observed for ferromagnetic materials with sizes larger than 10 nm [22]. The hysteresis loop is related to the pinning of magnetic domain walls at grain boundaries or impurities within the material. It can also be the result of intrinsic effects such as the magnetic anisotropy of the crystalline lattice [22.25].

As apparent from Fig. 3, the saturation magnetization of the magnetic nanoparticles is

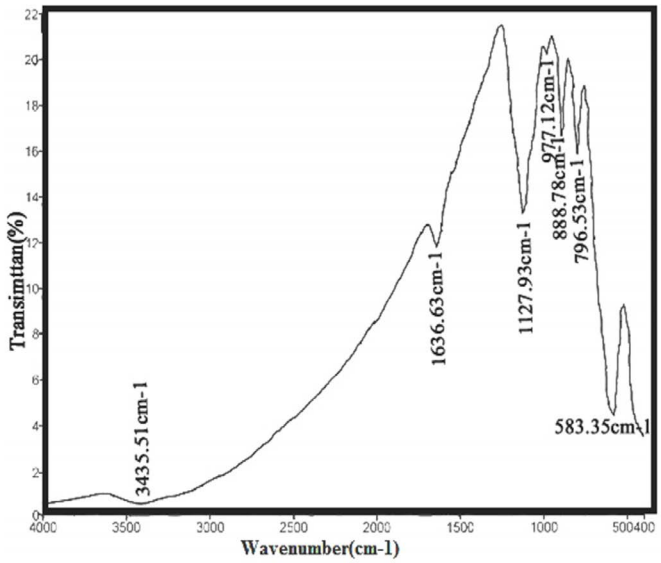

Fig. 2. FTIR spectrum of the sample 1: the synthesis of magnetite nanoparticles in electrochemical cells by adding NaOH

Table 2 . Serum level of AST, ALT, and ALP in rats treated with different doses of nanostructure (X ± SD, n = 10)

Groups	AST(UL^{-1})	ALT (UL^{-1})	ALP (UL^{-1})	BUN ($mgdL^{-1}$)	Creatinine ($mgdL^{-1}$)
Control	56.4 ± 6.3	51.2 ± 6.5	90 ± 12. 2	5.2± 1. 1	0.45±0.2
10 ppm day^{-1}	64.4 ± 9.6	50.5 ± 7.4	95.3 ± 10.4	6.6± 0.3	0.35 ± 0.3
100 ppmday^{-1}	63.1 ± 7.2	46.3 ± 8.5	99.6 ± 13. 4	5.8± 0.7	0.44 ± 0.2
NPs 1000 ppm day^{-1}	86.2*±10.4	64.9*± 17.6	106.3* ± 13.5	8.2*± 1.2	0.54 *± 0.5

* means significant difference to the control group ($p<0.05$).

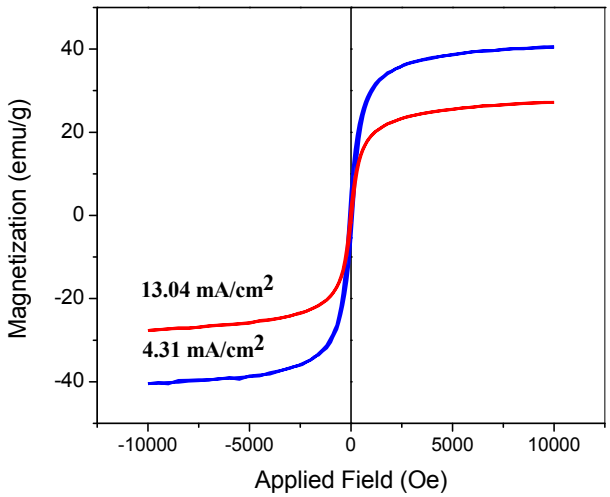

Fig. 3. Magnetization curve graph of 1 and 3 samples , magnetite nanoparticles produced by electrochemical method in current density and in presence of NaOH

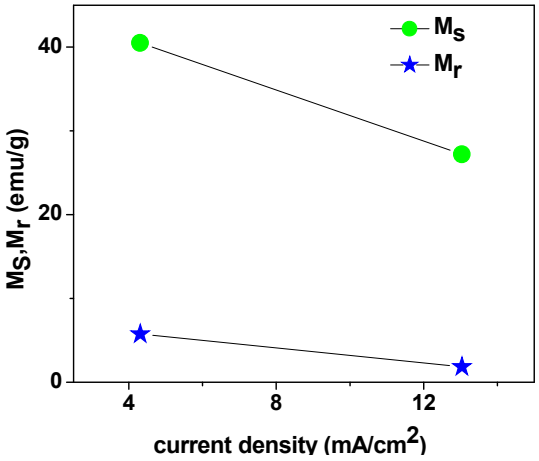

Fig. 4. Variations of saturation and remnance magnetization as a function of current density for magnetite nanoparticles of sample 1 and 3 produced by electrochemical method in presence of NaOH.

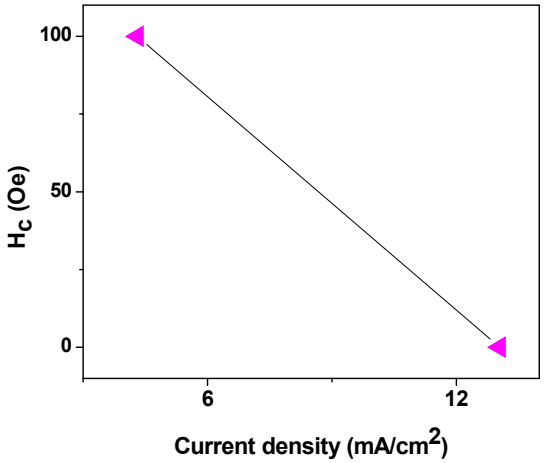

Fig. 5. Variations of coercivitiy as a function of current density for magnetite nanoparticles sample 1 and 3 produced by electrochemical method in presence of NaOH.

dependent on the current density, that takes the value of about 40.5 and 27.2 emu/g for the current densities of 4.31 and 13.04 mA/cm^2, respectively. The values are less than that of the Fe_3O_4 bulk materials (M_s = 92 emu/g) [26]. From the curve, it was also found that the coercivities have values of 0 and 100 Oe, which is in agreement with the expected value for randomly oriented, uniaxial, and non-interacting particles of magnetite [22].

As observed in the Fig. 4, slope of Ms and M_r vs current density plot is negative for sample 1 and 3. In the other words, the saturation and remnance magnetization reduced as the current density decreased. Fig.4 also shows that the slope variation of saturation magnetization as a function of current density is faster for magnetite nanoparticles sample 1 and 3 than that of remnance magnetization.

As the Fig. 5 demonstrates, slope of coercivitiy vs current density plot is negative for magnetite nanoparticles sample 1 and 3.

Since the graphs of sample 1 and 3 show magnetic remanence of 5.75 and 1.84, respectively, the ferromagnetic property of particles is well-confirmed.

The FESEM image of synthesized nano-sized magnetite particles is shown in Fig. 6 in the scale of a) 200 nm and b) 500 nm.

As it can be seen, the particles are quasi-spherical with a mean particle size of about 28 nm. The mean crystallite size estimated from the XRD is about 23

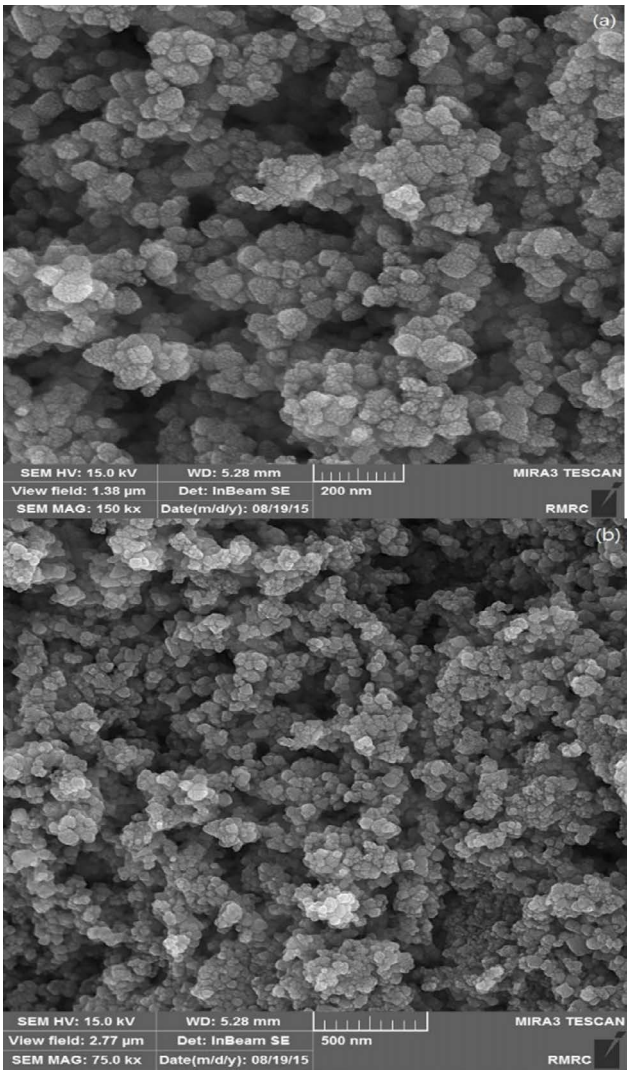

Fig.6. FESEM image of magnetite nanoparticles sample 1 in the scale of a) 200 nm, b) 500 nm.

nm, which is in agreement with the FESEM result.

The impact of current density on variation of average crystallite size of nanoparticles has been shown in Fig.7. It can be observed that the average crystallite size of nanomagnetites increased as the current density enhanced. This result agrees with previous result of the study by Fauziatul *et al.,* that reported the electrochemical generation of free-surfactant nanomagnetite [22].

Biological activity

Oral administration of nanoparticles was caused significant alterations to the levels of AST, ALT, and ALP in serum. Exposuring to 1000 ppm day^{-1} of nanostructure, significantly increased the serum level of AST, ALP and ALT compared to the control

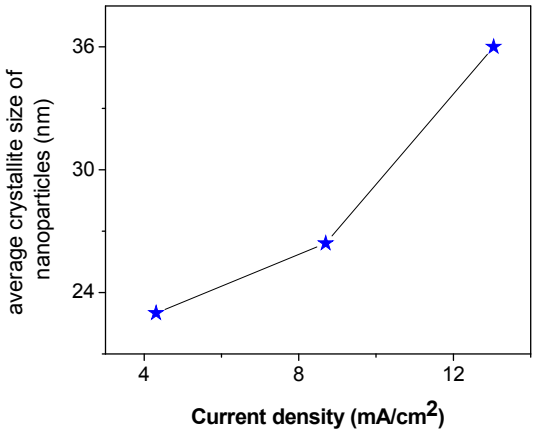

Fig.7. The effect of current density on particle size while the synthesis process was carried out in the electrolytic cells without page separators.

group. However, no significant change was detected in the groups treated with 10 and 100 ppmday^{-1} of nanostructure (P>0.05). There was a considerable increase in the serum level of creatinine and blood urea nitrogen (BUN) (p<0.05) in rats treated with high oral doses of the nanostructure [30-32]. Such increases may be related to the possible nephrotoxic potential of exposuring to the high doses of the nanostructure.

CONCLUSION

In conclusion,this study indicated that the synthesis of spherical-like iron oxide nanoparticles with a mean size of approximately 23-36 nm is achievedby a simple surfactant-free electrochemical method using an closed aqueous system in presence of NaOH. The analysis of x-ray diffraction pattern confirmed the formation of nano-sized particles of magnetite and also showed is the presensce of impurities in the form of FeOOH(a non-magnetic material). The superparamgnetic behavior of magnetite nanoparticles was confirmed by VSM technique based on current density-dependent saturation magnetization and coercivity. Our results indicated that the average crystallite size of nanoparticles increased as the result of the changes in current density. On the other hand, the coercivitiy, the saturation and remnance magnetization decreased as the current density increased. The biological activity study of nanoparticles on liver and kidney function in male wistar rats, showed the significant alterations to the levels of AST, ALT, and ALP in serum after oral administration of nanoparticles. However, in the other groups treated with 10 and 100 ppm day^{-1} nanostructure, no considerable change was observed. (P>0.05). Moreover, the serum level of creatinine and BUNwas significantly increased (p<0.05) in rats treated with high oral doses of the nanostructure.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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