Citric Acid Coated Iron Oxide Nanoparticles as Contrast Agent for Magnetic Resonance Imaging

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Abstract

Iron oxide nanoparticles (IONPs) were synthesized at varying pH (8 to 12) then subsequently coated with citric acid by co-precipitation as potential magnetic resonance imaging (MRI) contrast agents. The X-ray diffraction patterns confirmed that the structure of the as-synthesized powders is pure nanocrystalline magnetite Fe_3O_4 phase. FTIR analysis of citric acid-coated IONPs indicated the presence of peaks at 1357.70 and 1533.97 cm^{-1} , thus confirming the binding of citric acid on the surface of magnetite nanoparticles. Zeta potential measurements indicated that the citric acid-coated IONPs presents much-improved stability compared to bare IONPs; the Zeta potential absolute value is almost three times higher. Finally, citric acid-coated IONPs as a contrast agent for MRI clearly demonstrates their excellent ability to shorten T_2 and T_1 relaxation times by more than 94.7% and 67.9%, respectively. These results reveal that the citric acid-coated IONPs are excellent candidates for potential biomedical application primarily as MRI contrast agents.

1. Introduction

Iron oxide nanoparticles (IONPs) attracted great attention because of their outstanding and tunable chemical, optical and magnetic properties that are dependent on their morphology and size, chemical composition, and structural arrangement. These properties make them more suitable particularly for medical and pharmaceutical fields. Their nanometer size facilitates their movement (transport) within the body without disabling normal functions and may reach inaccessible locations. Moreover, IONPs possess additional distinctive characteristics such as availability, biochemical stability, biocompatibility, and the ability to be manufactured in various shapes and sizes [1, 2]. Specifically, IONPs exhibit superparamagnetic behavior and high surface area for biomedical applications, primarily in theragnostic (simultaneously as a contrast agent and magnetic fluid hyperthermia for cancer treatment) and targeted drug delivery. They provide a substantial imaging contrast in magnetic resonance imaging (MRI) by picking up the resonance absorption and consequently the relaxation process is accelerated. In drug delivery, the magnetic properties of IONPs can be manipulated using an external magnetic field so that the therapeutic procedure can be performed at the specified locations/organs [3–5].

Various methods have been adopted for the synthesis of IONPs such as thermal decomposition, co-precipitation, hydrothermal, and reverse micelles. Both thermal decomposition and hydrothermal methods have advantages for the production of unilateral IONPs, besides being flexible and adjustable [6]. It is possible to adopt thermal decomposition of iron five-carbonyl in the presence of oleic acid at 100°C . Also, it could be oxidized using tri-diethyl to produce highly crystalline and monodispersed maghemite nanoparticles. However, high reaction temperatures limited their applicability to biomedical applications [7]. Besides, such methods are complex and require very high temperatures, compared to the co-precipitation method which is commonly used to produce water-soluble and biocompatible IONPs. The as-prepared IONPs with a suitable surface functionality are effective for biomedical applications [8, 9]. .

Although IONPs exhibit interesting and controllable magnetic properties, they have high tendency to agglomerate as consequence prevent particles' surface functionalization with biocompatible entities such as citric acid. IONPs with sizes in the 1–100 nm can be either ferromagnetic or superparamagnetic (ability to have zero magnetism in the absence of external magnetic field). Superparamagnetic order occurs when the size of

the magnetic domain is so small that the surrounding thermal energy is sufficient to induce the free rotation of the crystals, known as Brownian motion [10, 11].

Extensive research in the literature focused on the preparation and understanding the magnetic behavior of IONPs. However, the reported synthesis routes faced challenges of producing suitable sized, shaped, structured and monodispersed IONPs with high magnetic moments. Although, several review papers have presented in-depth and breadth discussion and comparison on the synthesis of IONPs, most focused on the chemical methods. The synthetic routes can be classified into three: physical, chemical and biological methods. The chemical method is the most cited method of producing IONPs [6]. To prevent agglomeration, the as-synthesized IONPs are subsequently stabilized via peptization effect or steric stabilization. The use of several peptizing agents such as tetramethyl ammonium hydroxide, perchloric acid, citric acid, nitric acid and acrylic acid have been reported [12].

Several authors reported one pot synthesis process that combines both co-precipitation and stabilization procedure. This process involves additional stabilizing or peptizing agents such as citric acid, oleic acid, or a polymer during the co-precipitation process [13, 14]. IONPs of 10–100 nm can be synthesized by controlling the reaction conditions such as temperature, pH, stirring speed, aging time and ionic strength of the medium [15–17]. The saturated magnetization of IONPs produced via this method decreases by decreasing the particle size [18].

Sol-Gel method is also known as chemical solution deposition method which involves conversion of the precursor solution into an inorganic solid by chemical means [19]. This approach is an appropriate wet chemical method to synthesize metal oxide nanostructures with specific properties [20].

It is important to highlight that the behavior of IONPs in addition to their large magnetic susceptibility causes microscopic field heterogeneity and activation of proton removal in the presence of an external magnetic field. Therefore, it can be used as MRI probe (contrast agent) to shorten T_2 and T_1 relaxation times of the neighboring regions [21]. In this study, highly stable and monodispersed IONPs coated with citric acid are synthesized by one-pot co-precipitation method. Particular emphasis is devoted to examining the sensitivity of IONPs as MRI contrast agent in agarose gel phantom.

2. Materials And Methods

2.1 Samples preparation

Fe_3O_4 nanoparticles were synthesized by co-precipitation method of ferrous (Fe^{+2}) and ferric (Fe^{+3}) chlorides with NaCl and sodium hydroxide (NaOH). NaCl solution was prepared by adding 11.688 g of NaCl to 200 ml of deionized water (DIW). $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ (0.795 g) and $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ (1.297 g) were dissolved in 40 ml of DIW under nitrogen gas. Following the complete dissolution of the mixture at room temperature, 25 ml of 1.5 M of sodium hydroxide was added dropwise to the reaction mixture, under continuous vigorous stirring at 500 rpm for 2 hours. Only the pH value was varied from 8 to 12 by controlling the added amount of sodium hydroxide.

To prevent agglomeration of Fe_3O_4 nanoparticles and guarantee a high state of stability, 1.5 g of citric acid dissolved in 150 ml of DIW was added to the mixture described above for the sample prepared at pH 10, then

the new mixture was stirred for 15 min. at 70°C. Fe₃O₄ nanoparticles coated with citric acid were collected through a permanent magnet and thoroughly rinsed twice with distilled water to eliminate unreactive or inert impurities.

2.2 Samples characterizations

The crystalline structure of the as-prepared IONPs was checked by X-ray diffraction (XRD) using PANalytical Xpert PRO MRD PW 3040 diffractometers equipped with CuK α radiation source ($\lambda = 1.5418 \text{ \AA}$). The structural bonding and functional groups were determined by Fourier transformed infrared (FTIR) using the Perkin ElmerGX-1FTIR spectrophotometer. Zeta potential measurement was performed using Malvern instrument (Nano ZS model, ZEN 3600). The chemical composition was determined by atomic absorption spectroscopy (Shimadzu AAS-6650).

2.3 MRI measurements

For Magnetic Resonance measurements, IONPs synthesized at pH 10 and coated with citric acid was chosen. MRI phantoms were prepared from Agarose gel “Nutrient Agar” by dissolving 1 g of agarose in 100 ml of distilled water at 80°C. When fully dissolved, the agarose gel mixture was subsequently simmered down to 50°C. To prepare optimized IONPs sample, the concentration of 1 mg/mL was diluted to (0.5, 0.25, 0.125 mg/mL) and then centrifuged. The diluted samples were poured into glass vials containing 3 ml of 1wt% Agar gel solution. The solutions were mixed well to achieve homogeneity and then kept cold until hardened. Finally, five agarose gel phantoms containing different concentrations of citric acid coated IONPs (Blank sample 0, 0.125, 0.25, 0.5 and 1 mg/mL) were prepared for MRI measurements.

3. Results And Discussion

3.1 X-ray diffraction analysis

XRD patterns of the final powders are shown in Fig. 1. The XRD patterns exhibit well-defined broad and relatively low intensity peaks typical characteristics' of nanocrystalline phases. The peaks located at 2θ 30.17°, 35.43°, 43.45°, 57.07° and 63.16° are indexed as (220), (311), (400), (511) and (440) reflections corresponding to the magnetite Fe₃O₄ phase with a cubic structure (Fd-3m space group), in agreement with JCPDS card No. 01-075-0449 for magnetite. No additional peaks can be detected hence confirming the purity of the as-prepared nanoparticles irrespective of pH value, besides it indicates that citric acid coating does not induce any phase transformation of bare Fe₃O₄ [22–24].

The average crystallite size (D in nm) has been determined by means of the well-known Debye-Scherrer's formula given by [25]:

$$D = K \lambda / \beta_{hkl} \cos \theta$$

where K is grain shape constant (0.89 for spherical shape), $\lambda = 0.154056 \text{ nm}$ is the wavelength of XRD beam, β_{hkl} is the full width at half maximum for the diffraction peak (radian), and θ is the diffraction angle. The effect of pH on the size of IONPs is presented in Table 1. It is clear that the crystallite size increases gradually as the pH of the solution increases from 8 to 12. However, at the pH 10, it is interesting to note that crystallite size decrease after citric acid; i.e., 11.91 to 10.19 nm respectively. According to the obtained results, IONPs prepared

at pH 10 manifest a dark black color and are more stable compared to the remaining set of IONPs, hence were subsequently coated with it was citric acid and used for MRI measurements.

Table 1
Effect of pH of solution on the crystallite size of Fe₃O₄ nanoparticles.

pH of Solution	Size of Fe ₃ O ₄ Nanoparticles (nm)
8	10.55
9	11.18
10	11.91
11	13.44
12	13.93
10 (coated)	10.19

3.2 FTIR analysis

The as-prepared IONPs are characterized by FTIR in order to detect functional groups and characterize the existing covalent bonding. Figure 2 presents a typical FTIR spectrum recorded for IONPs prepared at pH 10 and coated with citric acid. The strong absorption band centered at 418.85 cm⁻¹ is assigned to the Fe-O bond, which confirms the formation of Fe₃O₄ phase [26]. The peak at 1357.70 cm⁻¹ can be ascribed to the asymmetric stretching of C–O from the carboxylic group [27]. Citric acid binds to Fe₃O₄ particles’ surface through carboxylate group. The 1600 cm⁻¹ peak assigned to the C = O vibration (symmetric stretching) from the COOH group of citric-acid (CA) shifts to an intense band at about 1533.97 cm⁻¹ for citric acid coated Fe₃O₄, hence confirming the binding of a citric acid on the surface of magnetite. A broad and intense band at 3185.70 cm⁻¹ can be assigned to the structural OH groups as well as to the traces of molecular water and citric acid.

3.3 Zeta potential analysis

Colloidal stability in aqueous medium is one of the most important characteristics associated with biomedical applications of nanoparticles [28, 29]. Zeta potential measurement offers a key role in understanding the stability of nanoparticles [30]. From the Zeta potential diagrams presented in Fig. 3, it can be concluded that the stability of the citric acid coated IONPs presents a much improved stability compared to bare IONPs, since the Zeta potential absolute value is almost three times higher; i.e. -26.6 ± 3.57 and - 9.51 ± 3.8 mV, respectively [31].

3.4 Atomic absorption spectroscopy analysis

Iron (Fe) concentration of Fe₃O₄ nanoparticles coated with citric acid was determined AAS. The standard solution was analyzed and the absorbance was measured at the wavelength of 248.3 nm [32]. The standard curve of Fe concentration with absorbance at 248.3 nm was plotted in Fig. 4. Using the calibration curve, the concentration of Fe in Fe₃O₄ nanoparticles coated with citric acid was found to be 155.4 ppm (0.1552 mg/mL)

which is safe and acceptable and does not present any toxicity threshold sensitive for medical applications including MRI [32].

3.5 Magnetic resonance measurements

Five samples of citric acid coated IONPs with different concentration of Fe were prepared to investigate their potential as contrast agent in MRI. To obtain T_1 and T_2 values, the images of agarose gel phantoms containing different solutions of Fe and blank sample were imported into image J software package and the signal intensities were measured. The images of the samples were obtained using spin-echo sequence device. For the relaxation time T_1 , the values of the echo-time (T_E) are fixed at 10 ms and that of the repetition time (T_R) is varied as 100, 150, 200, 300, 600, 1200, 2400, 4800 and 8000 ms. For the relaxation time T_2 , the values of T_R are fixed at 1000 ms and that of T_E is varied as 10, 15, 20, 30, 60, 120 and 200 ms. The other parameters are kept fixed at field of view (FOV) 40 mm with an image matrix of 256x256 pixels, slice thickness 3 mm, number of cuts of 3, and distance between slices is 0 mm.

The relaxation times T_1 and T_2 for various Fe concentrations were obtained using single exponential fit intensity against T_R and T_E . The values of T_1 for the samples with 0, 0.125, 0.25, 0.5, and 1.0 mg/ml Fe are found to be 3157.66, 2318.39, 2015.49, 1543.56, and 1013.44 ms, respectively. The relaxation time T_1 decreases gradually and substantially (~ 68%) from 3157.66 to 1013.44 ms with the increase in the concentration of Fe from 0 to 1 mg/ml. The results are summarized in Table 2 and plotted in Fig. 5.

Table 2
The signal intensities at fixed T_E (10 ms) and different T_R (ms) between 100 and 8000 ms. The T_1 (ms) recovery time of each phantom with different concentration of IONPs coated with citric acid is obtained from curve fitting.

TR(ms)		100	150	200	300	600	1200	2400	4800	8000	T_1 (ms)
Intensity	Blank sample	89	106	156	222	419	811	1360	1927	2331	3157.66
	(0 mg/ml)										
	(0.125mg/ml)	111	110	184	261	547	895	1430	1939	2172	2318.39
	(0.25 mg/ml)	105	129	202	311	554	957	1535	1931	2135	2015.49
	(0.5 mg/ml)	116	140	219	314	581	988	1384	1679	1795	1543.56
	(1 mg/ml)	121	152	210	327	540	840	1067	1194	1214	1013.44

Similarly, the values of T_2 for the samples with 0, 0.125, 0.25, 0.5 and 1 mg/ml Fe are found to be 173.61, 47.86, 32.40, 19.48 and 9.03 ms, respectively. The relaxation time T_2 decreases gradually and significantly (~ 95%) from 173.61 to 9.03 ms with the increase in the Fe concentration from 0 to 1 mg/ml. The results are summarized in Table 3 and plotted in Fig. 6. Thus, for both T_1 and T_2 -weighted images, it is observed that both T_1 and T_2 values decrease with the increase in Fe concentration, and this decrease is more significant for T_2 .

Table 3

The signal intensities at fixed T_R (1000 ms) and different T_E between 10 and 200 ms. The T_2 (ms) recovery time of each phantom with different concentration of IONPs coated with citric acid is obtained from curve fitting.

	TE (ms)	10	15	20	30	60	120	200	T_2 (ms)
Intensity	Blank sample	703	679	642	582	494	358	250	173.61
	(0 mg/ml)								
	(0.125 mg/ml)	785	677	645	502	268	81	43	47.86
	(0.25 mg/ml)	840	716	585	439	192	46	0	32.40
	(0.5 mg/ml)	838	632	480	305	79	0	0	19.48
	(1 mg/ml)	744	452	228	79	32	0	0	9.03

The relaxation rates (r_1 and r_2) are usually used to measure the enhancement efficiency of the contrast agent. To quantify the MRI contrast efficiency of citric acid coated IONPs, the r_1 and r_2 relaxation rates were obtained by measuring of relaxation times of different concentrations and plotting the inverse relaxation times (relaxation rates) versus different Fe concentrations, according to the following equation [33]:

$$1/T_{i,obs} = 1/T_{i,d} + r_i [M] \quad (i = 1, 2) \quad (2)$$

where $1/T_{i,obs}$ denotes the measured solvent relaxation rate in the presence of a contrast agent, $1/T_{i,d}$ is the solvent relaxation rate in the absence of a contrast agent, $[M]$ represents the contrast agent concentration, and r_i is defined as the relaxation rate in $\text{mM}^{-1} \cdot \text{s}^{-1}$. The relaxation times ($1/T_1$ and $1/T_2$) were plotted as a function of Fe concentration as shown in Fig. 8. As determined from the curve fitting in Fig. 8, the values of r_1 and r_2 of citric acid coated IONPs are found to be 0.654 and $103.02 \text{ mM}^{-1} \cdot \text{s}^{-1}$, respectively. The obtained results are due to the smaller distance between water molecules and magnetic core than that in the presence of nanoparticles. The smaller water molecules interact chemically with the magnetite particles' surface, and hence the water molecules cannot go close enough to the core as in the case of naked particles.

Reduced motion means that a higher proportion of HOH nuclei will experience both static fields (reducing T_2) and interactions near the Larmor frequency (reducing T_1) [34]. The r_2/r_1 ratio is a key parameter to determine the efficiency of citric acid coated IONPs for MRI contrast agents [35]. Its value is found as 157.52, demonstrating that IONPs can be efficient as a T_2 contrast agents.

Compared to other iron oxide-based contrast agents of similar sizes [36, 37], the values of r_2 of $103.02 \text{ mM}^{-1} \cdot \text{s}^{-1}$ and relaxivity ratio of 157.02 obtained in this study for IONPs coated with citric acid are found to be very high which is a clear indication for devising the same as an efficient T_2 contrast agent.

The obtained results indicate that r_1 and r_2 values depend on surface modification (for instance coating with citric acid), the particles size of IONPs core, and the degree of aggregation [38]. The observed high r_2/r_1 ratios show that IONPs coated with citric acid can be considered as potential candidate to be utilized as MRI contrast

agents in the future. We found that the particles synthesized in this work exhibit higher relaxivity ratios due to the presence of negative surface charges.

5. Conclusion

Pure and stable IONPs were produced using a cost-effective co-precipitation method with optimum properties obtained at pH 10 which particle size about 11.91 nm. Subsequent coating with citric acid as a capping agent has been performed in order to optimize surface functionalization and increase further the stability of IONPs. From the physicochemical analyses of the as-synthesized IONPs coated with citric acid by means of XRD, FTIR, AAS, and Zeta potential, the obtained results demonstrated that the method was successful for preparing high quality IONPs with a good colloidal stability. Due to the high magnetic moment of the as-synthesized IONPs coated with citric acid, the sensitivity of the composite particles as MRI contrast agent was evaluated. The results revealed that citric acid coated IONPs rapidly shortens T_2 and T_1 relaxation times of tissue equivalent Agarose phantom by 94.7% and 67.9%, respectively. Moreover, the efficiency evaluated from the relaxivity ratio indicated IONPs coated with citric acid were highly effective for MRI T_2 contrast agent with r_2/r_1 value of 157.52. Finally, it was concluded that the sensitivity of IONPs coated with citric acid prepared demonstrate a potential as a candidate for MRI contrast agent.

Declarations

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Competing Interests

"The authors have no relevant financial or non-financial interests to disclose."

Author Contributions

"Authors contributed equally to the manuscript and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript."

Data Availability

"The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request."

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Figures

Figure 1

X-ray diffraction patterns of Fe₃O₄ nanoparticles at different pH values.

Figure 2

FTIR Spectrum of Fe_3O_4 nanoparticles prepared at pH 10 and coated with citric acid.

Figure 3

Zeta Potential distribution for the coated and non-coated Fe₃O₄ nanoparticles prepared at pH 10.

Figure 4

Atomic absorption spectrum Fe_3O_4 nanoparticles prepared at pH 10 and coated with citric acid.

Figure 5

The intensity as a function of echo time T_R for different Fe concentrations.

Figure 6

The intensity as a function of repetition time T_E for different Fe concentrations.

(a)

(b)

Figure 7

The relaxivity graph of (a) $1/T_1$ and (b) $1/T_2$ against different Fe concentrations.