Rapid Synthesis of Aqueous-Phase Magnetite Nanoparticles by Atmospheric Pressure Non-Thermal Microplasma and their Application in Magnetic Resonance Imaging

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Here, we present a novel approach for producing Fe$_3$O$_4$ nanoparticles (NPs) in liquid by a non-thermal argon microplasma. The Fe$_3$O$_4$ NPs can be generated in minutes without any surfactant with an average size of $12.5 \pm 2.4$ nm. They show good aqueous-phase stability and excellent superparamagnetic properties with a saturation magnetization of $60.1$ emu g$^{-1}$ and a small coercivity ($<10$ Oe). In vitro magnetic resonance imaging study shows efficient imaging contrast characteristics of these NPs. These Fe$_3$O$_4$ NPs can be further decorated with polyethylene glycol (PEG) by adding 5% PEG into the electrolyte, which resulting in an average diameter of $19.2 \pm 2.7$ nm. This facile, fast, environmentally- and economical preparation strategy of magnetite NPs may be extended to the preparation of other metal oxide compound.

1. Introduction

Magnetic iron oxide nanoparticles (NPs) have found clinical and research applications due to their ability to produce contrast in $T_2$ and $T_2^*$-weighted magnetic resonance images.$^{[1-3]}$ These NPs have been extensively studied as potentially superior negative contrast compared with the most widely used chelated paramagnetic ions, due to their high susceptibilities, biocompatibility and lower toxicity.$^{[4]}$ In the past decades, nature-inspired or aqueous-phase synthetic iron oxide NPs, such as superparamagnetic iron oxide (SPIO) and related NPs (e.g. crosslinked iron oxide (CLIO), Feridex, Resovist, and Combidex) have served as contrast-enhancing probes for MRI.$^{[5]}$ To improve MR contrast effects and incorporate more versatile surface groups for advanced molecular imaging, researchers have been developing next generation NPs probes. Magnetite (Fe$_3$O$_4$) NPs have been the most promising and popular candidate due to their proven biocompatibility and low cost.$^{[6-9]}$ After serving their purposes in clinical diagnosis, Fe$_3$O$_4$ NPs can then be captured and disposed of by the liver without adverse effects.$^{[10]}$

Various chemical methods have been reported for the synthesis of Fe$_3$O$_4$ NPs, such as coprecipitation, thermal decomposition, microemulsion, and hydrothermal
The coprecipitation technique is probably the simplest and the most efficient way to obtain magnetic NPs. However, it has limited control of the particle size distribution. Monodisperse NPs can essentially be synthesized through thermal decomposition of organometallic compounds in organic solvents containing stabilizing agents. This method however usually requires relatively higher temperatures and a fairly complex operation. The microemulsion method employs a thermodynamically stable isotropic dispersion of two immiscible phases (water and oil) with the presence of surfactant(s). The surfactant molecules form a monolayer at the interface between the oil and water, and the reaction takes place in the microdroplets (typically 1–50 nm). The main advantage of the microemulsion system is that the size of the NPs can be controlled by modulating the size of the aqueous droplet core under room temperature. However, large amounts of solvents are used and the throughput of NPs is quite low compared to other methods. Hydrothermal synthesis works in a sealed container at high temperature (generally in the range of 130–250 °C) and high vapor pressure (generally from 0.3 to 4 MPa), which limits its application. Moreover, the solvent replacement process has to be carried out to transfer these organic-phase NPs into the aqueous phase, which usually results in size and shape variations and poor dispersion and stability. NPs prepared with these chemical methods are often synthesized in organic solvents, making them non-biocompatible. Synthesis of water soluble magnetite NPs that are suitable for biomedical applications remains a challenge.

Alternatively, many different plasma processes have been used for gas-phase NPs synthesis without chemical reducing agents or surfactants, including alternating current (AC), direct current (DC), radio frequency (RF), and microwave systems. Using these plasma reactors, one however cannot easily synthesize well-dispersed NPs, especially to define materials below approximately 10 nm in scale. In recent years, plasma–liquid interactions at atmospheric pressure and room temperature have drawn much attention as a novel nanoparticle synthesis method. By reducing the critical dimensions of the devices down to micrometer range enables the non-thermal plasma to be sustained at atmospheric pressure and room temperature. The high density of electrons (with energies in excess of 10 eV) present in these plasmas allows the direct reduction of metal cations in aqueous phase. These atmospheric pressure non-thermal plasmas can therefore be used as a “contactless” electrode to avoid the difficulty of NPs isolation from the electrodes. Recently, Richmonds et al. showed that microplasmas can electro-chemically reduce noble metal cations and generate noble metal (e.g. silver and gold) NPs at ambient conditions. Tokushige et al. reported the synthesis of Fe and FePt NPs in molten LiCl–KCl–CsCl under 1 atm of argon atmosphere using plasma-induced electrolysis. The preparation of the melt usually took place under vacuum and high temperature (298–498 °C), making it difficult for industrial application. Besides, synthesis of metal oxide NPs has met limitations associated with more complex reactions. In this paper, we report the synthesis and characterizations of magnetite NPs in aqueous phase by microplasmas.

2. Methods and Materials

2.1. Experiment Details

All chemicals were of analytical grade and were used without further purification. The experimental setup of the Fe3O4 NPs synthesis system is shown in Figure 1. The reaction was performed in a U-shaped electrochemical cell, which consisted of a microplasma cathode and a platinum (Pt) foil anode. A copper capillary tube (7 cm long with an inner diameter of 0.355 mm, KS Engineering, US) was positioned 1 mm above the surface of the solution and was pressurized with argon (Ar) gas (99.99%) at a constant flow rate of 50 SCCM (standard cubic centimeter per minute). The electrochemical cell was driven by a direct current negative-polarity high-voltage power supply (Matsuada AU5R120). To stabilize the discharge, a 5.1 kΩ ballast resistor was placed between the anode and the power supply. Since Magnetite (Fe3O4) involves mixed oxidation states Fe3+ (ferric) and Fe2+ (ferrous) of iron at a molar ratio of 2:1, 2 mM ferric chloride (FeCl3) and 1 mM ferrous chloride (FeCl2) mixture are used as the precursors with an initial pH set
to 5.0 by sodium hydroxide. Upon the application of the high DC voltage, a microplasma filament formed in the space between the end of the copper capillary tube and the surface of the solution. The microplasma discharge was sustained at a voltage around 400 V with an operating current of 13 mA. The solution under the microplasma turned dark within seconds, indicating the effective formation of Fe₃O₄ NPs.

### 2.2. Characterization

Transmission electron microscope (TEM; FEI, Tecnai F-20, USA) was used to evaluate the size, shape and morphology of the resultant NPs, coupled with an selected-area electron diffraction (SAED) detector that was used to identify the crystal structure of the NPs. A drop of the NPs was applied to 300 mesh copper grid (coated with a continuous carbon support film) and dried at room temperature overnight. The sample was then analyzed in the TEM at an accelerating voltage of 200 kV. The synthesized NPs were separated by a magnet and dried at room temperature for 24 h. X-ray diffraction (XRD) pattern was acquired from these dried magnet and dried at room temperature for 24 h. X-ray voltage of 200 kV. The synthesized NPs were separated by a sample was then analyzed in the TEM at an accelerating voltage of 200 kV. The synthesized NPs were separated by an accelerating voltage of 200 kV.

### 2.3. MR Imaging

Suspensions of synthesized NPs were collected by a magnet and prepared in different concentrations in 2% agarose solution. Based on the amount of agarose solution and NPs, the iron concentrations were calculated as 0, 0.81, 1.63, 3.26, 6.25, and 13.03 mg mL⁻¹, respectively. The solutions were then injected into six wells of a 96-well cell culture plate (Corning Costar®). For magnetic properties measurements, the dried NPs powder was fixed by acrylic AB adhesive and cut into the size of mung beans. The measurement was carried out in a vibrating sample magnetometer (VSM, LDJ9400, LDJ Electronics, US) at room temperature. The magnetization of the encapsulated particles versus the applied field at room temperature (300 K) was measured with a maximum applied field of 10 000 Oe.

### 2.4. PEG coated Fe₃O₄ NPs

To generate PEG coated Fe₃O₄ NPs, 5% (mass ratio) PEG (Sinopharm, Molecular Weight = 4 000) was added to 100 mL deionized water, mixed with 2 mM ferric chloride and 1 mM ferrous chloride. The microplasma discharge current was sustained at 13 mA and voltage was around 420 V. Other conditions for PEG coated Fe₃O₄ NPs generation were the same as for bare Fe₃O₄ NPs generation. PEG coated Fe₃O₄ NPs were collected by magnet after 10 min reaction. The morphology and size distribution of these coated NPs were studied by TEM (FEI, Tecnai F-20, USA). The effectiveness of PEG surface coating was investigated by Fourier transform infrared spectroscopy (FTIR) using a Bio-Rad Digilab Division FTS-65A/896 Fourier transform infrared spectrometer equipped with a Harrick’s Meridian SplitPea single-reflection diamond attenuated total reflectance (ATR) accessory. The PEG coated Fe₃O₄ NPs were collected by magnet and washed three times with de-ionized water before dried for the FTIR experiments. The infrared spectra were recorded between 4000 and 400 cm⁻¹. The magnetization of the encapsulated particles versus the applied field at room temperature (300 K) was measured with a maximum applied field of 10 000 Oe.

### 3. Results and Discussion

#### 3.1. Synthesis of Fe₃O₄ NPs

When the cathode end of the electrochemical cell was open to the ambient, overoxidation of iron ions resulted in the production of α-Fe₂O₃ NPs in the solution, a material that is non-magnetic at room temperature. Therefore, Ar gas was used to fill the head space of the reaction apparatus through port B₁ (Figure 1). B₁ served as the cathode exhaust port. A match was lit above the open cathode end of the electrochemical cell. We waited for 0.5–1 min, until the match died out completely to confirm that most of the air was driven out of the headspace. Upon the application of a DC high voltage, a jet-like microplasma formed in the space between the end of the copper capillary tube and the surface of the solution. After a 10 min reaction, the synthesized Fe₃O₄ NPs were isolated from the solution by a 0.6 T magnet (isolation only took seconds) and dried overnight at room temperature. A total of 3.7 mg Fe₃O₄ NPs were collected.

The actual chemical reaction in the microplasma-solution system is rather complex. However, we believe that hydroxyl radicals were probably either produced via (i)
electron impact dissociative excitation of H₂O directly by high energy electrons from the plasma, at the plasma–liquid interface, or (ii) the formation of argon metastable by electron impact excitation in the gas phase, followed by the dissociative excitation of H₂O by argon metastables at the gas–liquid interface. These reactions are listed below together with further cascaded chemical reactions in the bulk liquid that led to the production of Fe₃O₄ NPs:

a). Production of hydroxyl radicals

\[ e_{\text{gas}} + H₂O \rightarrow OH^* + H^* \]  

(1) (Production of hydroxyl radicals, plasma–liquid interface reaction)

b). Production of hydroxyl radicals, gas–liquid interface reaction

\[ Ar + e_{\text{gas}} \rightarrow Ar^* \]  

(2) (Excitation of argon atoms, gas phase reaction)

\[ Ar^* + H₂O \rightarrow Ar + H + OH^* \]  

(3) (Production of hydroxyl radicals, gas–liquid interface reaction)

c). Fe₃O₄ NPs formation:

(1)OH^* + OH^* → H₂O₂

(Bulk liquid reaction)

(2)Fe²⁺ + H₂O₂ → Fe³⁺ + OH^* + OH^−

(Fenton Reaction, bulk liquid reaction)

(3)Fe³⁺ + e → Fe²⁺

(Reduction, gas–liquid interface reaction)

(4)Fe²⁺ + 2Fe³⁺ + 8OH^− → Fe₃O₄ + 4H₂O

(Formation of Fe₃O₄ nanoparticles, bulk liquid reaction)

The excessive electrons supplied by the microplasma cathode should also in principle be able to reduce the metal cations to zero-valent irons (ZVI), which may in turn be partially oxidized to Fe₃O₄. However, Patel et al. [29] pointed out that, free electrons that do not contribute to the electron impact excitation in the gas–liquid interface and reach the bulk of the liquid will thermalize and hydrate on sub-nanosecond timescales. It is more probable that the electron-induced reactions at the plasma–liquid interface promote cascaded chemistry leading to hydrogen peroxide, that further participate in the redox reactions in the bulk liquid.

The pH value of the ferrous and ferrite chlorite solution is essential for the generation of NPs in liquid. The original value of the pH was around 2–3 due to the hydrolysis of ferric/ferrite ions. Under such a condition, the synthesized Fe₃O₄ NPs quickly re-dissolved in the acidic solution. Stabilization of the Fe₃O₄ NPs in the solution was achieved only when the pH value was adjusted to around 5 by sodium hydroxide. This pH value is also favored by the Fenton reaction.[30] It is also worth noting that potassium iodide starch test paper placed near the anode exhaust port B₃ (Figure 1) became blue over time, indicating the formation of chlorine gas at the grounded platinum electrode. The pH value of the solution decreased accordingly near the Pt electrode, but hardly, if at all affected the solution near the microplasma cathode.

### 3.2. Characterization of Fe₃O₄ NPs

A TEM was used to evaluate the size, shape and morphology of the resultant NPs. As shown in Figure 2, the NPs are spherical in shape and have an average size of 12.5 ± 2.4 nm (based on 200 NPs). The histogram in Figure 2b shows the size distribution of the NPs. The dotted rings in the SAED pattern for these NPs shown in Figure 2c suggests that these NPs have good crystallinity. Analysis of the d-spacing of these rings suggests that the dotted rings represent the Bragg reflection of [111], [220], [311], [400], [422], [511], and [440] crystal planes, indicating that the produced Fe₃O₄ NPs have a standard face-centered cubic (fcc) spinel crystal structure.

Figure 2. a) Typical TEM image of the NPs; b) A histogram of the size distribution of NPs with average diameter 12.5 ± 2.4 nm; c) Electron-diffraction pattern of an ensemble of NPs; d) HRTEM image of a typical single crystal of Fe₃O₄ NP.
hematite, maghemite, and goethite). [33–35]

An XRD system was used to elucidate the crystal structure of the obtained NPs. As shown in Figure 3, the XRD pattern indicates that these NPs preserve the typical features of the magnetite spinel phase, with a series of characteristic peaks at 20 = 18.29°, 30.10°, 35.45°, 43.08°, 53.45°, 56.98°, 62.57°, 74.02°, and 89.68°, which correspond to the [111], [220], [311], [400], [422], [511], [440], [533], and [731] Bragg reflections, respectively. This is in agreement with the standard magnetite (Fe3O4) XRD JCPDS file (PDF No.65-3107) by comparison with the XRD patterns of other iron oxide compounds (Fe(OH)3, hematite, maghemite, and goethite). [31]

Magnetic hysteresis curve is shown in Figure 4. The saturation magnetization (Mₘ) of the synthesized NPs is 60.1 emu g⁻¹ and the coercivity is <10 Oe at room temperature. According to Wu et al., [36] experimental value for Mₘ in magnetic iron oxide NPs prepared by chemical method have been reported to span the 30–80 emu g⁻¹ range. This result suggests that the Fe3O4 NPs synthesized directly in liquid by the atmospheric pressure non-thermal microplasmas have excellent superparamagnetic property with a low saturation field and a high susceptibility, making them a promising rapid-responding MRI contrast agents.

3.3. MR Imaging

Over recent decades, Fe3O4 NPs have been intensively investigated as magnetic resonance T₂/T₂* spin–spin relaxation contrast agents. [1,2] In the presence of an externally applied magnetic field, inhomogeneity in the magnetic field was created by magnetic NPs that resulted in dephasing of the magnetic moments of protons. These changes result in the darkening of the corresponding area in T₂ MR images. [37] The degree of the T₂ contrast effect is typically represented by the spin–spin relaxation rate R₂, where higher values of R₂ result in a greater contrast effect. The relaxivity coefficient (r₂), which is obtained as the gradient of the plot of R₂ versus the molarity of magnetic atoms, is a standardized contrast enhancement indicator. Figure 5a shows the standard T₂-weighed MR images of the agarose solutions with the iron concentrations in the solution being 0, 0.81, 1.63, 3.26, 6.25, and 13.03 μg mL⁻¹, respectively. These images were taken with TR = 4000 ms and TE = 25 ms. Figure 5b shows the MR image signal intensities at different TE (25, 50, 75, and 100 ms) with increasing concentration of bare Fe3O4 NPs in the agarose solution. Solid lines represent the mono-exponential fitting curve. The relaxation rate, R₂ = 1/T₂, is linearly proportional to the iron concentration (as shown in Figure 5c). The relaxivity coefficient (r₂) of our magnetic NPs is calculated from Figure 5c as 404 mM⁻¹ s⁻¹. The r₂ value of other conventional types of NPs, such as Feridex, Resovist, Combidex or CLIO, is in the range of 50–150 mM⁻¹ s⁻¹. The higher value of r₂ is attributed to the higher local inhomogeneity induced by the magnetite NPs in the magnetic field. This suggests a better contrast property of our magnetic NPs than other types of NPs. They could therefore be used as a more sensitive MRI contrast agent. This.

3.4. PEG coated Fe3O4 NPs

The Fe3O4 NPs are usually incorporated with versatile surface groups for advanced molecular imaging. These naked Fe3O4 NPs generated by microplasmas can be easily decorated with many different functional groups by adding corresponding polymers (e.g. dextran, dendrimers,
polyethylene glycol (PEG), and polyethylene oxide) into the electrolyte. These polymer coatings are biocompatible and result in a long blood circulation time. The PEG coated \( \text{Fe}_3\text{O}_4 \) NPs were easily synthesized by microplasma in the presence of PEG under 10 min reaction time. The TEM image (Figure 6a) shows that the PEG-coated \( \text{Fe}_3\text{O}_4 \) NPs is monodisperse and the core particle size is increased to 19.2 \( \pm \) 2.7 nm (Figure 6b) compared to bare \( \text{Fe}_3\text{O}_4 \) NPs. On the other hand, the presence of nonmagnetic PEG layer on the surface of NPs can lead to decrease of \( M_s \) of the \( \text{Fe}_3\text{O}_4 \) NPs. Work on using these NPs for bio-imaging, biodetection, and drug delivery is underway and will be reported in our future papers.

Many researchers have shown that PEG worked as an ideal stabilizer by formation of hydrogen bonding between adjacent PEG coated on \( \text{Fe}_3\text{O}_4 \) NPs. In order to examine the coating effect of PEG to the NPs, Fourier transform infrared (FTIR) spectra of \( \text{Fe}_3\text{O}_4 \) NPs, PEG, and \( \text{Fe}_3\text{O}_4 \) NPs coated with PEG were recorded (Figure 7). The FTIR absorption for \( \text{Fe}_3\text{O}_4 \) NPs shows characteristic peaks at around 590 and 3400 cm\(^{-1}\), which can be assigned to the lattice absorption of the Fe—O bond vibration and the stretching vibration of OH groups on the surface of the \( \text{Fe}_3\text{O}_4 \) NPs. When using PEG as the dispersing agent, some new absorption peaks typical for PEG appear besides the peaks from the \( \text{Fe}_3\text{O}_4 \). The sharp peak at 1106 and 1342 cm\(^{-1}\) is from the \(-\text{C—O—C—}\) stretching (the associated hydroxyl groups). The peak at 2890 and 964 cm\(^{-1}\) can be attributed to \(-\text{CH}\) stretching vibrations and \(-\text{CH}\) out-of-plane bending vibrations, respectively. The \(-\text{C—O—C—}, -\text{CH}\) peaks are strong evidence that PEG covered the nanoparticle surface.

4. Conclusion

An atmospheric pressure non-thermal microplasma is used to replace the solid cathode in a conventional electro-chemical cell for water-soluble and biocompatible magnetite nanoparticle synthesis. Well-dispersed \( \text{Fe}_3\text{O}_4 \) NPs with an average size of 12.5 \( \pm \) 2.4 nm were produced in the solution under the right pH and argon gas buffer. These \( \text{Fe}_3\text{O}_4 \) NPs showed good contrast-enhancing effects for MR imaging. These naked \( \text{Fe}_3\text{O}_4 \) NPs can be easily decorated with PEG, forming PEG coated...
Application of a promising new method for the production of aqueous phase magnetite nanoparticles by microplasma. The simplicity of the apparatus and room temperature operation make this plasma–liquid interaction based approach a promising new method for the production of metal oxide NPs in liquid for various nano-technological applications.

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Fe3O4 NPs of an average diameter of 19.2 ± 2.7 nm. Although the Fe3O4 NP production rate is only ~0.37 mg min⁻¹, parallel operation of these microplasmas in the same cell or in multiple cells can significantly improve the throughput. The simplicity of the apparatus and room temperature operation make this plasma–liquid interaction based approach a promising new method for the production of metal oxide NPs in liquid for various nano-technological applications.