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Original Article

Surface Modification of Superparamagnetic Iron Oxide Nanoparticles by Argon Plasma for Medical Applications

Saleh Asghari [1], Mohammad Ali Mohammadi [2], Roya Julaei [2], Ramezan Ali Taheri [3*]

[1] Student Research Committee, Baqiyatallah University of Medical Sciences, Tehran, Iran
[2] Department of Atomic and Molecular Physics, Faculty of Physics, University of Tabriz, Tabriz, Iran
[3] Nanobiotechnology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran

Corresponding Author: Ramezan Ali Taheri, PhD, Assistant Professor, Nanobiotechnology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran. Tel: +98-9126110865, E-mail: taheri@bmsu.ac.ir

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Abstract

Introduction: Hyperthermia is rapidly becoming a clinical reality as a tool for the treatment of malignant disease but there are some major problems in the way of using superparamagnetic nanoparticles coated with polymer in medical applications. Modifying the magnetic nanoparticles without using surface coating is more appropriate. In this study, we presented a new physical technique, by surface treatment of nanoparticles with argon gas plasma, to modify the surface of nanoparticles for improving their crystal structure and magnetization.

Materials and Methods: In this study, Fe_3O_4 nanoparticles were synthesized using the co-precipitation method. The nanoparticles were then treated with plasma in a vacuum chamber. In this method, the Radio Frequency (RF) generator 13.56 MHz was used as a power supply and the plasma treatment was applied for 10 and 15 min.

Results: Due to the decrease in surface irregularities, the nanoparticle aggregation decreased and their colloidal stability increased. Moreover, with Value Stream Mapping (VSM) analysis the magnetism of the nanoparticles improved along with an increase of plasma power and plasma treatment time due to the reduced crystal defects and crystal growth. By using the AC magnetic field generator with a frequency of 92 kHz and amplitude of 125 Oe, results show that along with an increase of plasma power and plasma treatment time due to the increased magnetization and colloidal stability, heat generation by these nanoparticles increased in a ferrofluids system in the presence of AC magnetic field. In addition, the locking temperature of nanoparticles has also increased.

Conclusions: Our results suggest that the surface modification of Fe_3O_4 nanoparticles, using plasma treatment, is an appropriate candidate for some medical applications such as magnetic resonance imaging, drug delivery, and especially for magnetic hyperthermia.

Keywords: Surface Modification, Superparamagnetic, Iron Oxide Nanoparticles, Plasma Treatment, Hyperthermia, Medical Applications

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Introduction

In recent decades, there has been a special attention to the development of the Magnetic Nanoparticles (MNPs) for diagnosis and cancer targeted treatment and environmental means.[1-4] Also, due to biocompatibility, ultra-small size, chemical stability in physiological circumstances, and significant accumulation at the disease site, the Superparamagnetic Iron Oxide Nanoparticles (SPIONs) are more attended.[5,6] Moreover, these SPIONs are highly potential to act as a contrast agent in Magnetic Resonance Imaging (MRI), and can also generate heat in an AC magnetic field for the cancer-specific hyperthermia applications.[7,8] Moreover, MNPs are used as magnetically targeted carrier systems in drug delivery. Notably, the hyperthermia method using magnetic nanoparticles is considered as a promising approach to improve the treatment of certain cancers, due to reducing the dose of chemotherapy and having fewer side effects.[9] Also, advances in the performance of MNPs for local heating can be known as a good option to kill cancer cells without limiting penetration into the tissue. However, hyperthermia requires much improvement to destroy cancer cells completely. It is worth mentioning that heat generation by nanoparticles is dependent on particle size, crystallinity, and colloidal stability of nanoparticles.[10-14] Moreover, heat generation using MNPs is carried out in a ferrofluids system under an AC magnetic field in two ways as follows: (1) Brownian relaxation (particle rotation) and (2) Neel's spin relaxation (magnetic moment rotation). The SPIONs are suspended in proper carrier liquids, which are commonly called ferrofluids. In this regard, each particle only has a single magnetic domain that can be treated as small thermally agitated magnets in the carrier liquid. Besides, combining normal liquid behavior with superparamagnetic properties is one of the ferrofluids features. Accordingly, these properties of the ferrofluids enable us to use it for many technical applications.[15-18] The average size of these nanoparticles for medical application has been reported to be 10 nm. By paying attention to the size of cells, viruses, proteins, and genes; infiltration and even labeling the cells and tissues are important. Because of a high-surface/volume ratio as well as strong magnetic dipole-dipole attractions, MNPs tend to be aggregated. Therefore, it is vital to control the surface modification of these particles.[19-21] Correspondingly, such modifications can be made by creating a suitable coating of polymers and biocompatible molecules such as Polyethylene Glycol (PEG), Polyvinyl Alcohol (PVA), poly (acrylic acid), dextran, and chitosan on nanoparticles.[7,22-25]

There are two major problems in the way of using superparamagnetic nanoparticles coated with polymer in medical applications. Firstly, these coated nanoparticles lose their chemical stability inside the body by absorbing blood plasma proteins and increasing particle size. Secondly, in some cases, the polymer coating of nanoparticles is removed in the physiological environment.[26,27]

A more appropriate solution is modifying the MNPs without using surface coating. In this study, we presented a new physical technique, by surface treatment of nanoparticles with argon gas plasma, to modify the surface of nanoparticles for improving their crystal structure and magnetization. Therefore, due to the crystalline order, the aggregation of nanoparticles decreases and heat generation in the hyperthermia system increases.

Materials and Methods

Synthesis of Fe_3O_4 Nanoparticles

In this study, Fe_3O_4 nanoparticles were synthesized using the co-precipitation method. Also, starting materials including Iron chloride and sodium hydroxide (NaOH) of analytical grades were supplied by Merck Inc. (Darmstadt, Germany). To synthesize Fe_3O_4 nanoparticles, the iron salts Fe (II) ($FeCl_2.4H_2O$) and Fe (III), ($FeCl_3.6H_2O$) with a molar ratio (Fe^{3+}: Fe^{2+} = 1:2) were dissolved in 50 ml of deionized water at 80 °C. Afterward, sodium hydroxide (NaOH) was added to the solution as a precipitator. As soon as the precipitator was added, a black solution was formed and the nanoparticles grew for 30 min at 80 °C. The nanoparticles were then dried for one week at room temperature and consequently a black powder was prepared.

Plasma Treatment

For plasma treatment, the Fe_3O_4 powders were placed in a plasma reactor (Figure 1). The plasma reactor includes a cylindrical glass and a capillary copper tube with an external diameter of 2 mm which is wrapped around a cylinder used as an electrode. Moreover, in order to reduce the contamination, the reactor was evacuated to 0.01 Torr using a rotary pump. Notably, working gas and pressure of the reactor are argon and 0.25 Torr. In this study, the RF generator 13.56 MHz was used as a power supply and the plasma treatment was applied for 10 and 15 min.

Characterization

The crystallographic analysis of the obtained samples was accomplished using a XRD (Siemens D-500, Philips) with specification Cu-kα X-ray and wavelength of 1.54 Å. The Vibrating Sample Magnetometer (VSM- Kashan) with a maximum field of 15 kOe was also applied at room temperature to investigate the effect of plasma treatment on the magnetic properties of Fe_3O_4 Nanoparticles. To determine morphology and size distribution, the Scanning Electron Microscopy (SEM-LEO 1430VP) was used. The heat generated in the hyperthermia test was measured using AC magnetic field generator with a frequency of 92 kHz and amplitude of 125 Oe.

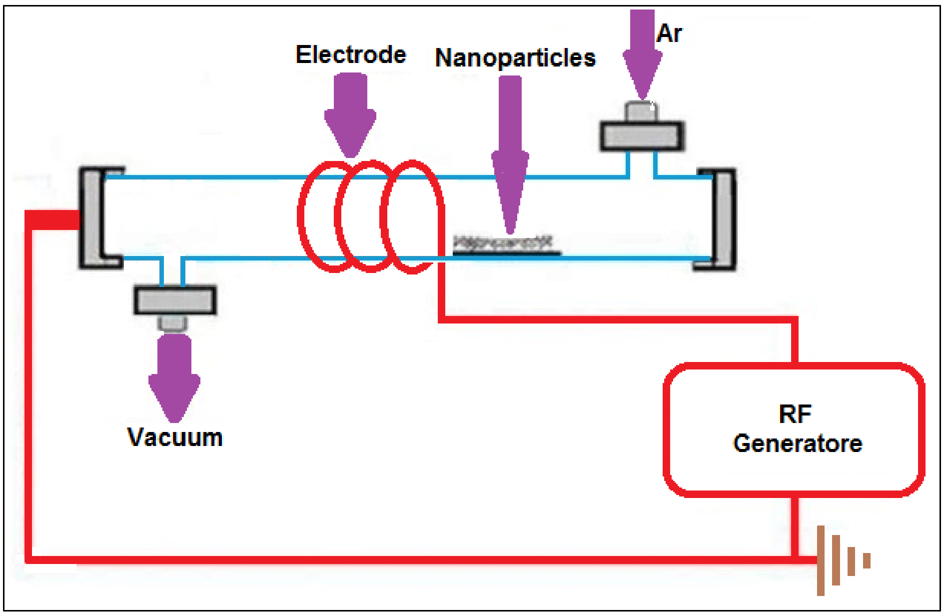

Figure 1. The Experimental Set-up for The RF Plasma Reactor.

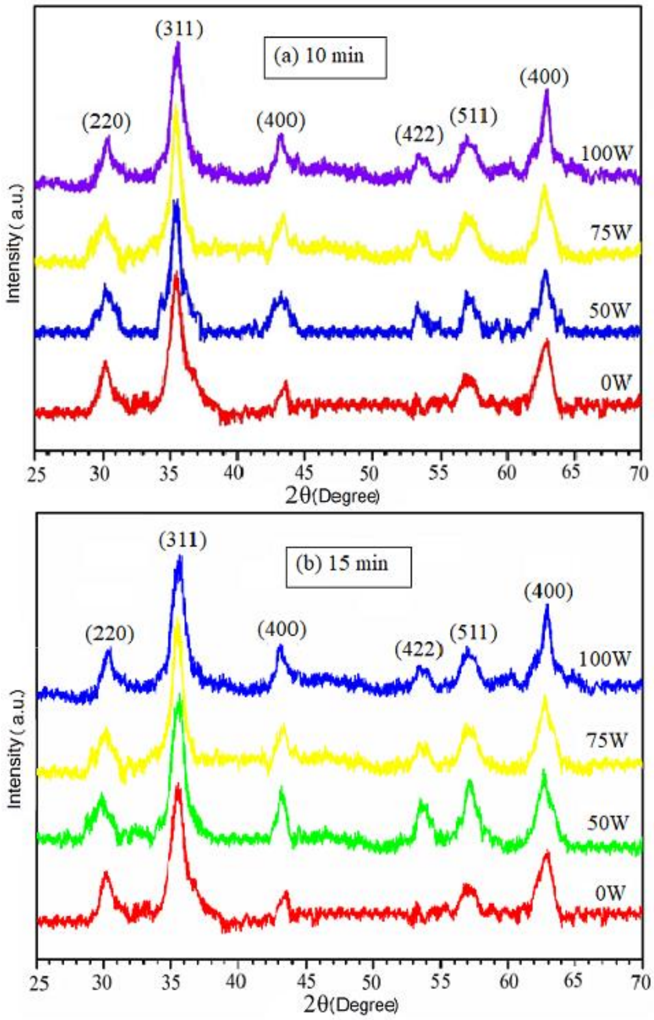

Figure 2. X-ray Diffraction Patterns of Fe_3O_4 Nanoparticles Under the Plasma Treatment for **(a)** 10 min, and **(b)** 15 min.

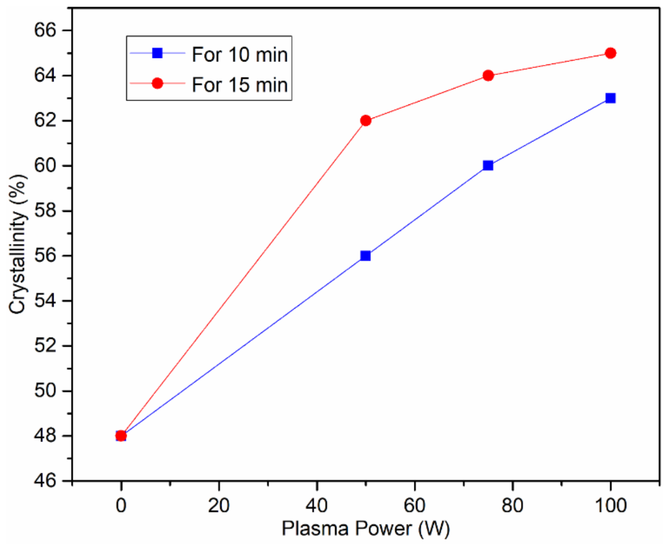

Figure 3. The Effect of Increasing Plasma Power and Treatment Time on the Crystallinity of the Nanoparticles.

Results and Discussion

Figure 2 shows the X-ray diffraction patterns of Fe_3O_4 nanoparticles before and after the plasma treatment. Using the Scherrer equation, the average crystallite size was obtained to be 10.56 nm for the Fe_3O_4 nanoparticles without any modification. For those samples that were modified by argon gas plasma, along with increasing plasma power, the average crystallite size increased up to 12.6 nm in 10 min. Accordingly, this can be attributed to the increase in surface energy of nanoparticles due to the collision of energetic ions by argon gas. In terms of the principle of energy minimization, the system tends to reduce energy and also decrease the surface-to-volume ratio. As a result, the crystals that were smaller, were stuck together and then formed larger crystals. Therefore, the energy of the system was minimized by the increased size of the nanoparticles. When the plasma treatment time was set to 15 min, the crystallite size increased up to 13.5 nm along with an increase of plasma power. So, this indicates that, as the plasma treatment time increases, the size of the nanoparticles also increases.

According to Figure 3 obtained from the X-ray diffraction results, it can be seen that with increasing plasma power, the crystallinity of the nanoparticles also increases. Correspondingly, this can be attributed to the modification of the crystalline defects of nanoparticles as well as the growth of crystals under the plasma treatment. This means that with increasing surface energy under the plasma treatment, incompletely synthesized crystals join together to form a more regular crystal. Notably, when the treatment time was increased (15 min), the greatest shift in the crystallinity percentage occurred at 50 W and with an increase of plasma power, this percentage did not change significantly. In this case, it can be stated that, with increasing the plasma treatment time, the highest percentage of crystallinity occurs at low power. Moreover, with increasing plasma power due to the excessive impact of high energy ions on the nanoparticles, the growth rate of the crystals reduces.

Figure 4 shows the magnetic hysteresis loop of the nanoparticles at different powers and times before and after the plasma treatment. In the samples that were under the plasma treatment for 10 min, with increasing plasma power, saturation magnetization increased from 52.4 to 60.7 emu/g. The increase magnetic ability of the nanoparticles after the plasma treatment can be ascribed to the increase in size and crystallinity percentage. In addition, when the nanoparticles were treated for 15 min, the saturation magnetization increased from 52.4 to 64.5 emu/g. Accordingly, this means that the increment in the duration of treatment increased the magnetic ability.

As shown in Figure 4, the magnetic remanence of the nano-particles slightly decreased after the plasma treatment, which means that the plasma treatment has enhanced superparamagnetic properties.

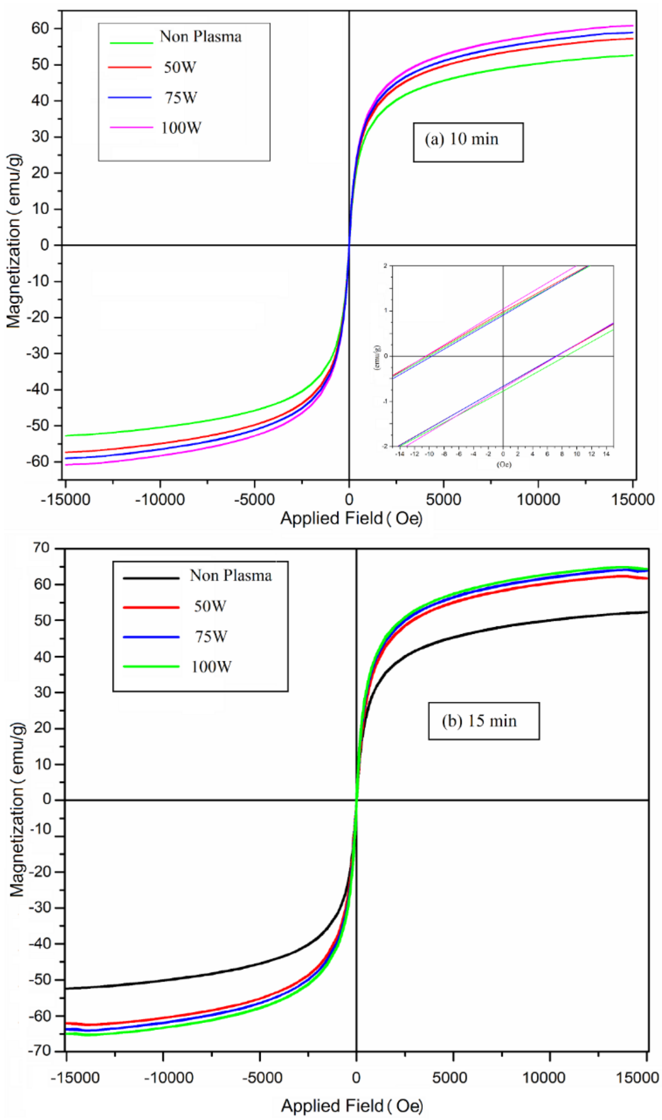

Figure 4. The Magnetic Hysteresis Loop of the Nanoparticles at Different Powers and Times Before and After the Plasma Treatment.

The colloidal stability of the nanoparticles was investigated in a ferrofluids system for a 10-day period. As shown in Figure 5, the treated samples maintained their colloidal stability well after these 10 days.

Figure 6 shows the SEM image of the nanoparticles before and after the plasma treatment. As indicated in these images, the crystal structure of the nanoparticles has improved by the plasma after observing surface modification and the nanocrystals separately. Due to the increased crystalline order and the reduced surface irregularities, the nanoparticles were better dispersed in water. Also, the nanoparticle aggregation has also reduced, which confirm the colloidal stability.

To study heat generation of the samples, we put the suspensions of nanoparticles inside a copper coil and then applied AC magnetic field to the samples (Figure 7) for approximately 30 min. To calculate the generated heat, the Specific Absorption Rate equation (SAR) was used as follows (Eq. 1):

Figure 5. Colloidal Stability of Nanoparticles for **(a)** the Untreated Sample, **(b)**, **(c)**, and **(d)** the Samples Treated with 50, 75, and 100W (15 min).

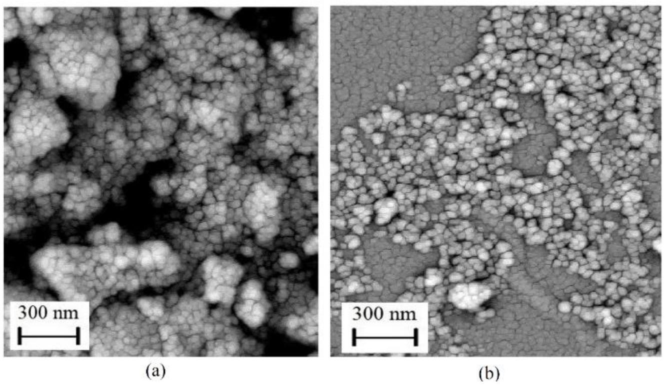

Figure 6. The SEM Image of the Nanoparticles Before and After the Plasma Treatment (15 min).

$$SAR = \frac{Ms}{Mn} C \frac{\Delta T}{\Delta t} \quad (1)$$

Where Ms is the mass of suspension including distilled water and nanoparticles, Mn is the mass of Fe_3O_4 nanoparticles, C the specific heat capacity of distilled water, and $\frac{\Delta T}{\Delta t}$ is the initial linear slope of the ΔT-time curves.

In this study, suspension of nanoparticles was prepared by dissolving 0.1 g of nanoparticles in 10 ml distilled water for 20 min using an ultrasonic device. Afterward, the obtained samples were placed under the magnetic field of the AC

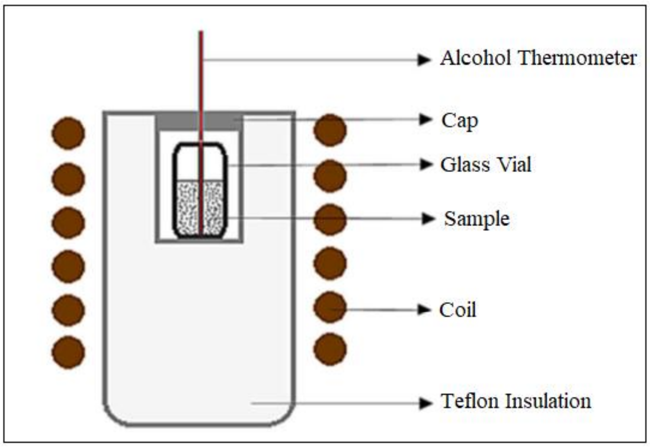

Figure 7. The Experimental Set-up for Hyperthermia.

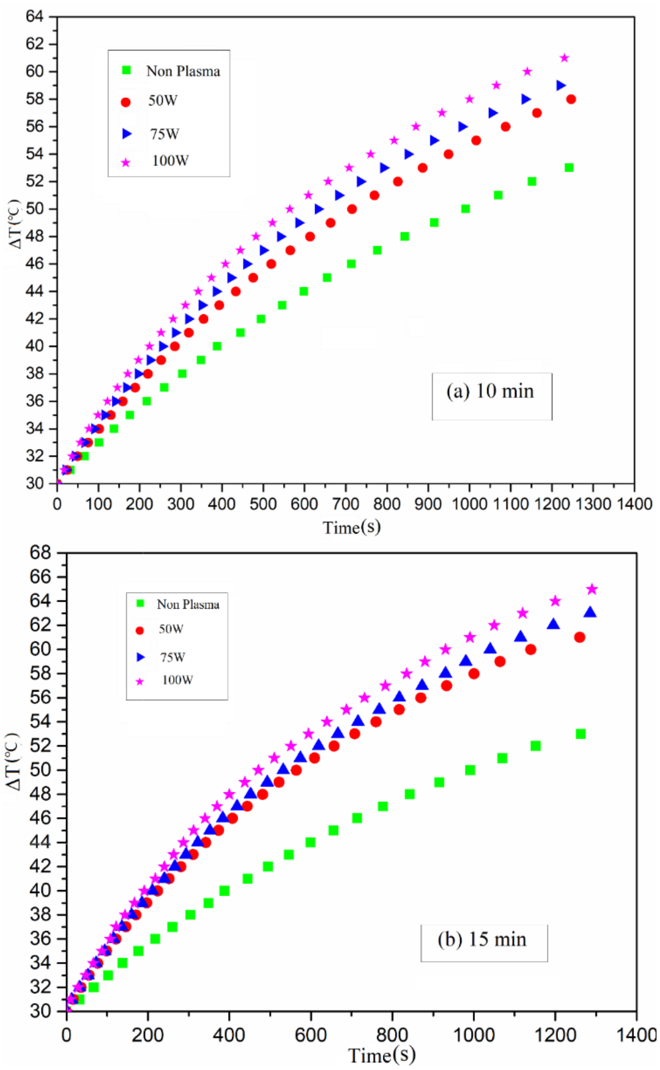

Figure 8. The Variation of the Hyperthermia Results of Fe_3O_4 Nanoparticles Under the Plasma Treatment for **(a)** 10 min, and **(b)** 15 min.

with a frequency of 92 KHz and amplitude of 125 Oe.

Figure 8 shows the variation of hyperthermia results of the samples before and after the plasma treatment. The SAR regarding the curve slope in the range of 0-360 s, for the untreated samples, and those samples treated with 50, 75, and 100W was 11.45, 16.1, 18.8, and 19.6 w/g, respectively. In the untreated sample, with an increase of time, the heat generation increased up to 53 °C. When the nanoparticles were treated by argon gas plasma for 10 min, with increasing plasma power, heat generated elevated and reached 61 °C. According to Figure 8b, it can be seen that increasing the plasma treatment time increased the blocking temperature of the nanoparticles by 13 °C (53-66 °C).

Conclusion

In this study, the effect of plasma treatment on Fe_3O_4 magnetic nanoparticles was investigated. According to the results of X-ray diffraction, the plasma treatment increased the crystallinity of the nanoparticles and also decreased the crystalline defects. With an increase of plasma power and treatment time due to the increased particle size and crystalline order, the magnetization ability of the nanoparticles also increased. The colloidal stability of nanoparticles improved after the plasma treatment due to the reduced surface irregularities. As a result, with increasing plasma power and plasma treatment time due to the increased magnetization and colloidal stability, heat generation by these nanoparticles increased in a ferrofluids system in the presence of AC magnetic field. Moreover, the locking temperature of nanoparticles has also increased. Our results confirm that the plasma-modified nanoparticles can be considered as a good option for medical applications, especially for magnetic hyperthermia.

Authors' Contributions

MAM and RAT devised the project, the main conceptual ideas and proof outline. SA and RJ performed the experiments. SA wrote the manuscript and RAT and MAM revised it. All authors provided critical feedback and helped shape the research, analysis and finalizing the manuscript.

Conflict of Interest Disclosures

The authors declare that they have no conflicts interest.

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