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Functionalized Superparamagnetic Iron Oxide Nanoparticles for Magnetic Fluid Hyperthermia Application in Cancer Treatment

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Abstract

In this work, we report the synthesis of functionalized superparamagnetic iron oxide nanoparticles (SPIONS) to use them as nano medicine to treat cancer via magnetic fluid hyperthermia-based therapy. For this purpose, we initially synthesize iron oxide nanoparticles by chemical co-precipitation method with its surface modified by pluronic (F-127) polymer. The synthesized SPIONS were investigated for their structure, morphology and magnetic properties. The crystal structure and morphology were characterized by X-ray diffraction technique (XRD) and Transmission electron microscopy (TEM), the interaction between pluronic (F-127) and Fe_3O_4 nanoparticles were characterized by Fourier transform infrared spectroscopy (FTIR). The average particle size of Fe_3O_4 sample was ~8.95 nm. The magnetic measurements revealed that, as prepared magnetic nanoparticles are superparamagnetic in nature and show high saturation magnetizations ~106 emu/gm for 10 K and 87 emu/gm for 300 K which is one of the important features of iron oxide for their use in hyperthermia therapy. The heat dissipation ability of Fe_3O_4 nanoparticles was determined using induction heating system. The biocompatibility of Fe_3O_4 was determined by measuring their cytotoxicity.

Keywords: Superparamagnetic iron oxide nanoparticles (SPIONS), Biocompatibility, cytotoxicity.

Introduction

Among the various nanoscale materials been investigated for biomedical use, magnetic nanoparticles have attained a significant attention, since it has diverse applications in biomedicine.

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This type of nanoparticles consists of metallic, bimetallic and superparamagnetic iron oxide-based nanoparticles (SPIONS). The latter has attracted a much attention due to its potential use in biomedical applications like cell separation [1], magnetic resonance imaging as a contrast agent [2], targeted drug delivery [3] and localized magnetic hyperthermia, since it has low toxicity and good biocompatibility. Magnetic hyperthermia is a cancer treatment wherein magnetic nanoparticles were locally injected into the cancer or tumor tissues that can be heated to the desired temperature of about 45 °C using an externally alternating magnetic field. The method was first used by Gilchrist et al. since 50 years ago [4]. Before this time, several works had been carried out to this subject were reported [5-7]. It was found that superparamagnetic Fe_3O_4 nanoparticles coated with biocompatible layer is appropriate for hyperthermia since it have large magnetic moment, high magnetic specific loss power (SLP), and non-toxicity. In addition, for superparamagnetic nanoparticles magnetic hysteresis is lost, the Neel and Brownian losses becomes dominant hence the specific loss power (SLP) of magnetic nanoparticles strongly depends on the alternating magnetic field as well as structure and nature of the particles such as particle size, anisotropy constant, saturation magnetization and surface modification [8-10]. Instead of this, it is important in hyperthermia to control the saturation temperature in the range of 42 – 47 °C to destroy the tumor cells with minimal damage to the healthy cells and restricts negative side effects. The purpose of this research work was preparing superparamagnetic Fe_3O_4 nanoparticles with high saturation magnetization to increase the SLP and focus on the heating ability with the concentration magnetic nanoparticles at alternating magnetic field.

Methodology

Materials and reagents

Ferrous chloride tetrahydrate ($\geq 99\%$), Ferric chloride hexahydrate ($\geq 99\%$) , Diisopropyl amine (DIPA) ($\geq 98\%$), were purchase by Loba Chemie. Hydrochloric acid (analytical grade) was used for synthesis purpose. Double distilled water was used throughout the work. All the reagents were used without further purification.

1. Synthesis of Fe_3O_4 nanoparticles

Superparamagnetic Fe_3O_4 nanoparticles were prepared by aqueous chemical co-precipitation method using DIPA as a base. In which 30 mmol of $FeCl_3.6H_2O$ and 15 mmol of $FeCl_2.4H_2O$ were dissolved in 50 ml of 0.5 M HCl solution. This solution was then quickly added to 250 ml of 3.0 M DIPA solution (pH 11-12) at room temperature with continuous mechanical stirring. A black precipitate was formed and stirring was continued for an hour at 80 °C. In addition to this, the precipitate was separated magnetically and washed several times with double distilled water. Finally, the obtained precipitate was dried at room temperature.

2. Coating Particles

The prepared nanoparticles were mixed in 2 ml of chloroform on vigorous mechanical stirring of about 20 min at room temperature. The resultant solution was added in 25 ml of aqueous solution of pluronic (F-127). The obtained mixture is stirred at 80 °C till water and chloroform gets evaporated.

Results and Discussion

Structural Investigation

1. X-ray Diffraction (XRD)

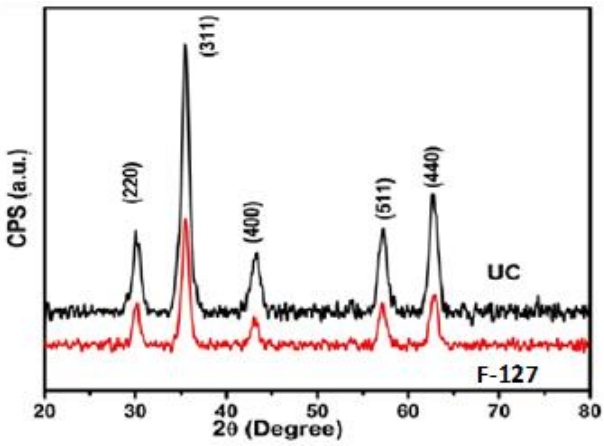


Fig 1: XRD patterns of uncoated and pluronic (F-127) coated nanoparticles (NPs)

Figure 1 shows XRD patterns of as prepared and Pluronic (F-127) coated magnetic nanoparticles. The XRD measurements were carried out using Bruker Axs D8 Advance diffractometer with Cu Kα-radiations (λ= 1.5418Å) with Ni filter over a range of 20-80°. The crystalline phase identified was iron oxide (Fe_3O_4) (Magnetite). The sharp peaks in XRD pattern indicates that the polycrystalline nature of the sample. The main reflection peaks are observed with the (hkl) values of (220), (311), (400), (422), (511) and (440). The values are then well matched with the JCPDS file number 19-0629, which corresponds to the bulk Fe_3O_4 phase [11]. The average crystallite size (t) of the particle is calculated using Debye Scherrer formula given by eq (1). The average crystallite size was found to be ~ 11 nm.

$$t = \frac{0.9\,\lambda}{\beta\cos\theta} \qquad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Where, β is the full width at half maximum (FWHM), K is the constant so called shaping factor which is 0.9 for spherical particles, λ is the wavelength, θ is the Bragg diffraction angle.

2. Fourier Transform Infrared Spectroscopy (FTIR)

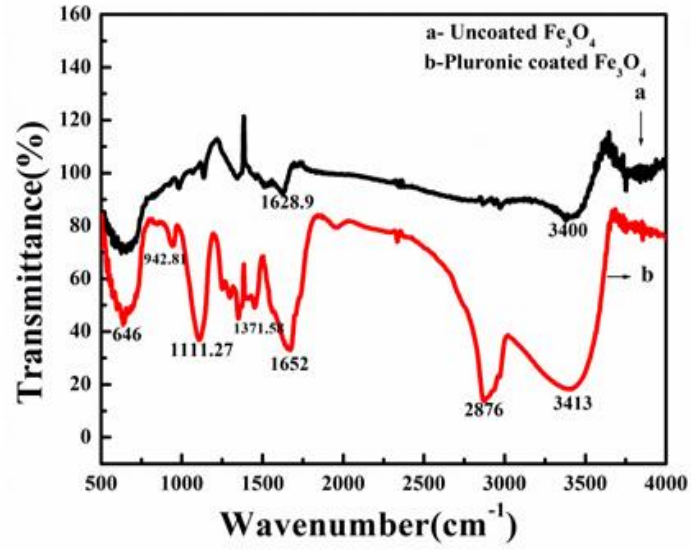


Fig 2: FTIR spectra of Fe_3O_4 NPs (a) Uncoated NPs (b) Pluronic coated NPs

FTIR spectra of Fe_3O_4 NPs asynthesized by the coating agent as well as the uncoated nanoparticles are shown in fig. 2. For the Fe_3O_4 Nps, the band at 646 cm^{-1} corresponds to the bending vibration of Fe^{3+}-O^{2-} in the tetrahedral complexes [11,12]. Additionally, the peaks at 1628 cm^{-1} and 3400 cm^{-1} cans be attributed to the stretching vibrations of the O-H group due to adsorbed water on the Fe_3O_4 NPs. For the spectra of the sample coated with Pluronic (F-127), new four bands appear at 942.81, 1111.27, 1371, 1652, 2876 and 3413 cm^{-1}. The band at 942.81 and 1111.27 cm^{-1} are assigned to the CH_2 rocking and characteristic C-O-C stretch of Pluronic coated NPs [13]. The bands appeared at 2876 and 1371 cm^{-1} can be ascribed to symmetric and stretching vibration of C-H and C-C-N respectively [14]. The five new bands demonstrate that, the coating agents are bonded to the surface of the magnetic nanoparticles.

3. Thermogravimetric analysis

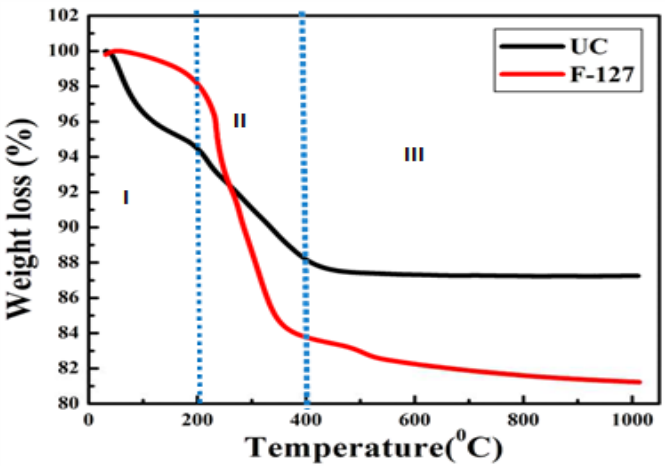
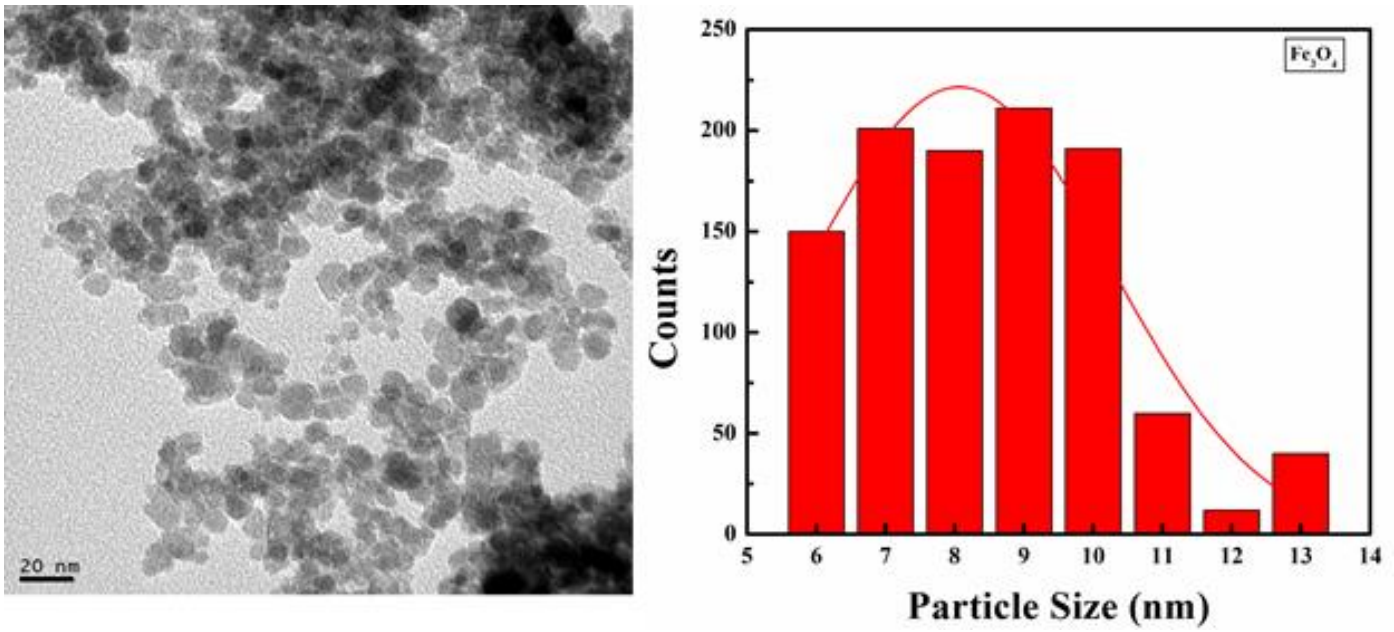


Fig 3 Thermal stability of uncoated and Pluronic (F-127) coated Fe_3O_4 NPs

The thermogram of uncoated as well as Pluronic (F-127) coated nanoparticles is shown in fig 3. From fig, it is observed that there are three main weight loss processes occurred. The initial weight loss at temperature below 150 °C refers to the evaporation of adsorbed water. The uncoated particle shows that the weight loss in this range about ~ 4%. The second and major weight loss of about ~12% occurs from ~200 to 400 °C, due to desorption of DIPA from the surface of the Fe_3O_4. The increase in boiling temperature of DIPA is due to the physical adsorption of multilayered DIPA on the surface of Fe_3O_4 NPs. This

strong desorption temperature indicates the strong bonding of DIPA on the surface of NPs.

However, in the case of coated NPs, ~3% weight loss is observed in the first region due to the evaporation of water from the surface. From 200 to 400 °C, ~15% weight loss is observed which is ascribed to the decomposition temperature of Pluronic (F-127) [15]. This shows that Pluronic (F-127) coated Fe_3O_4 NPs shows more weight loss as compared to uncoated Fe_3O_4 NPs. The small hump in the III[rd] region, above 400°C, indicates that desorption of carboneous matter from the surface of Fe_3O_4. From TGA it is evidenced that around 7% pluronic (F-127) is present on the surface of Fe_3O_4 NPs.



(a) **(b)**

Fig 4: (a) TEM image of Fe_3O_4 and (b) particle size distribution

4. Transmission Electron microscopy (TEM)

Fig 4 shows the TEM picture of as-synthesized Fe_3O_4 NPs. From TEM picture it, is observed that, all NPs are nearly spherical in nature, well distributed and slightly in aggregated form. This agglomeration is due to the high reactivity of the synthesized particles [16]. Histogram of the average particle size was generated from TEM images using image J software. The average size of the NPs would be ~8 nm.

5. Magnetic Properties

Fig 5 shows the temperature dependence hysteresis magnetization of as-synthesized Fe_3O_4 NPs at 10 K and 300 K.

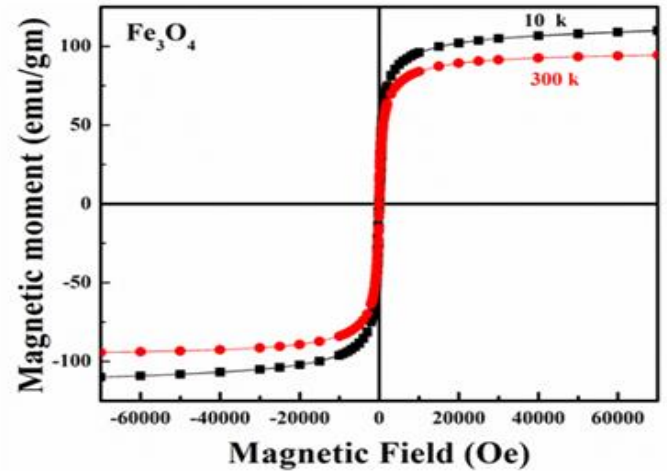


Fig 5: Magnetic properties of Fe_3O_4 NPs synthesized at 90 °C

From fig 5, it is observed that sample exhibit the superparamagnetic behavior which indicates that no hysteresis loop and possess zero remanence and zero coercivity. The saturation values of 10 K and 300 K are 106 emu/g and 92 emu/g respectively, which are higher than that of those were reported in the literature [17]**.** The magnetization at 10 K is higher than that of 300 K which is due to finite size effect associated with surface disorder and spin rearrangement imposed due to change in size of NPs below critical diameter.

6. Induction heating Studies

Self-heating temperature rise characteristics of uncoated and pluronic coated Fe_3O_4 nanoparticles at suspension of 5 and 10 mg mL^{-1} in distilled water at different amplitudes (20-30 kA m^{-1}) for 10 min were measured. In fig 1.6, gradual rise in temperature with increase in applied field amplitude is observed. For both the tested suspensions (5 and 10 mg mL^{-1}) of pluronic coated NPs, applied fields at 150 A were not sufficient to cause heating up to the hyperthermia temperature i.e upto 42 °C [18]. From the graph it is observed that, temperature rise goes on increasing with increasing the field amplitude. As the field increases, the time required to reach the hyperthermia temperature decreases. As can be seen from the figure, for the suspension of 10 mg mL^{-1} the time required to reach the threshold temperature for hyperthermia is lower compared to the 5 mg mL^{-1} and varies as a function of externally applied field. This may be due to the increase in exchange coupling energy due to increase in dipole dipole interactions in the presence of higher concentration of magnetic material in the suspension.

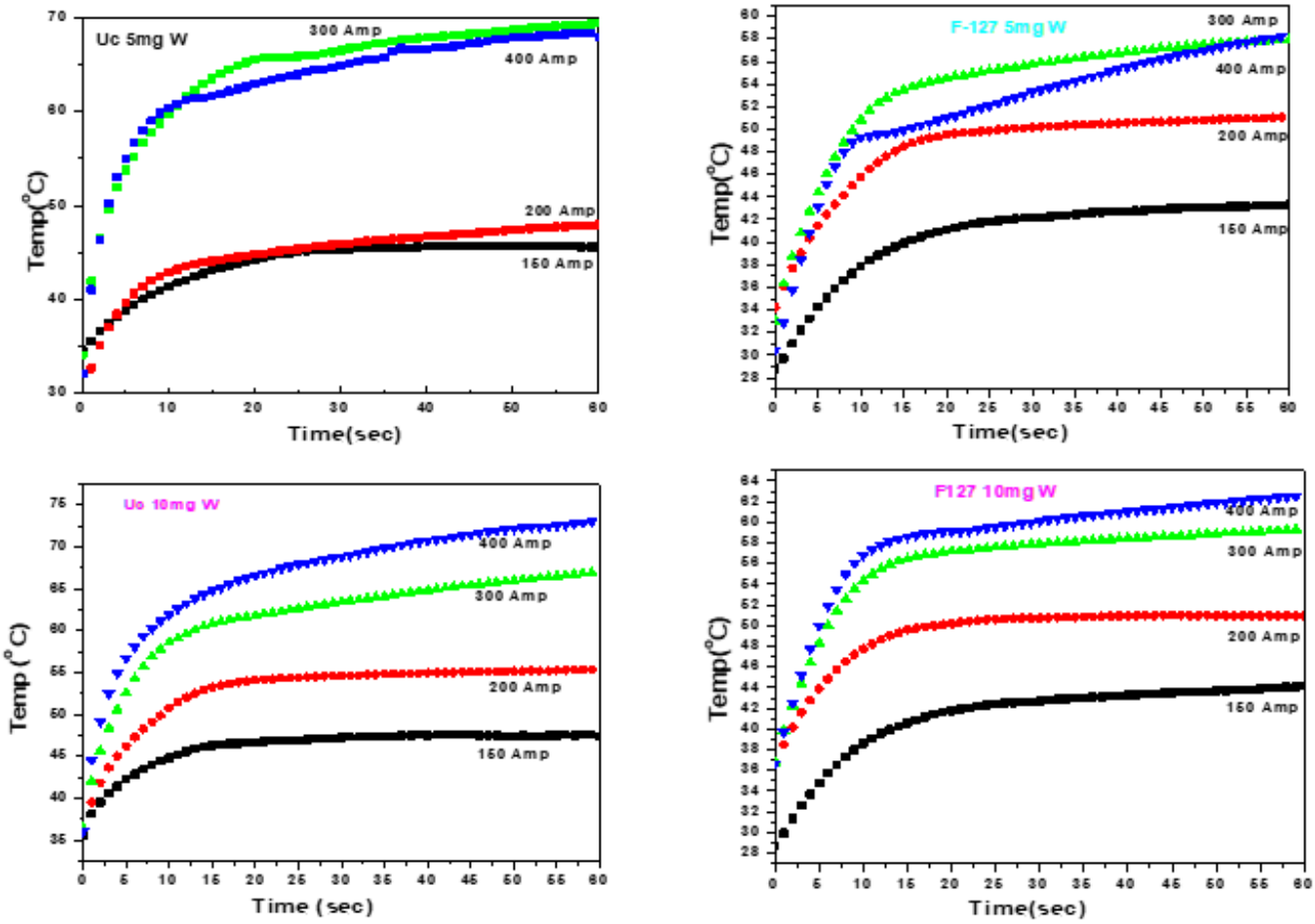


Fig 6: temperature versus time curve of uncoated and pluronic (F-127) coated NPs in water.

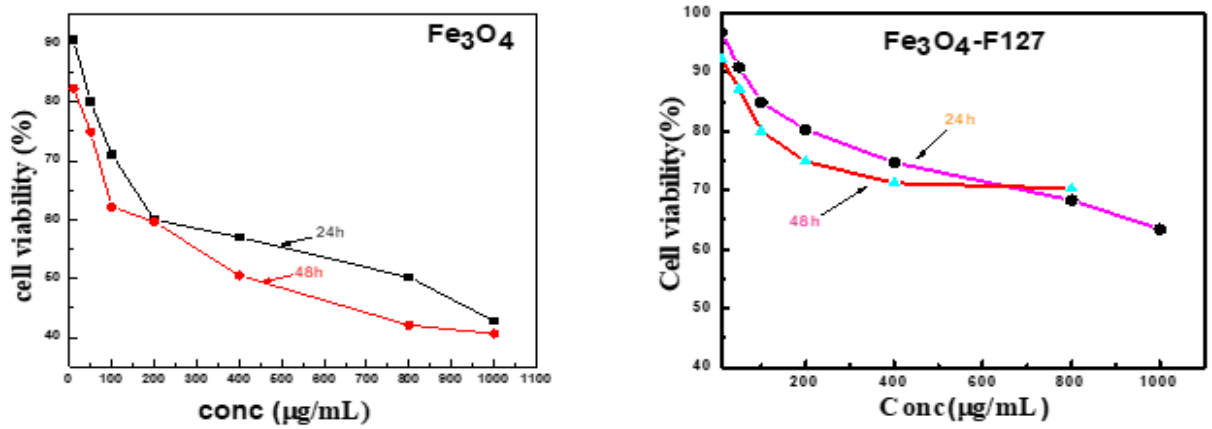


Fig 7: Cell viability of uncoated and Pluronic (F-127) coated nanoparticles

7. Cytotoxicity Study

Fig 7, shows the effect of Pluronic coated Fe_3O_4 NPs on the cell viability (%) of MCF-7 cells for 24 h and 48 h incubation time. From the graph, it is observed that, the uncoated Fe_3O_4 NPs caused ~ 50% cell death at the concentration of 800 µg/mL for 24 h. Instead of this, for the pluronic (F-127) coated NPs, 50% cell death occurred at relatively higher dose concentration more than 1000 µg/mL [19]**.** The pluronic (F-127) coated NPs have no major effect on cell viability at any of the concentrations tested which indicates that coating of polymer effectively improves the cytotoxicity of the Fe_3O_4 NPs. This may be due to the improved biocompatibility after surface functionalization [20]**.** From the cytotoxicity data, it is observed that, the cell viability gradually decreases with increasing the magnetic nanoparticles concentration and incubation time for samples. For pluronic (F-127) coated Fe_3O_4 NPs, at a concentration of 800 µg/mL and 1000 µg/mL the cell viability increases which is due to cell proliferation. The toxicity of Fe_3O_4 nanoparticles is reduced by biocompatible coating of pluronic (F-127) polymer on the surface ofthe Fe_3O_4 nanoparticles. These results imply that pluronic coated nanoparticles can be used for biomedical applications. More specific and refined studies regarding the cytotoxicity of the nanoparticles in presence of external magnetic field are currently under study.

Conclusion

Present work investigates the effect of capping agent on the surface behavior of iron oxide nanoparticles and its implication in cytotoxic effects on MCF-7 cell line for hyperthermia therapy application. Iron oxide nanoparticles with average size around 8-9 nm are synthesized by simple co-precipitation method using DIPA as a base. An x-ray diffraction and FT-IR result indicates the phase formation and presence of pluronic on the surface of Fe_3O_4 NPs. The weight loss with the variation of temperature was studied from the TGA graph. From magnetic measurements, it was confirmed that sample exhibit superparamagnetic behavior at room temperature with saturation magnetization 92 emu/g. Induction heating ability of these nanoparticles shows reasonable temperature rise under the application of external applied AC magnetic field. In vitro cytotoxicity evaluations based on MTT assay showed that coating of pluronic (F-127) polymer on the surface of NPs effectively improved their cytocompatibilty.

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