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Generation of drugs coated iron nanoparticles through high energy ball milling

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The iron nanoparticles coated with oleic acid and drugs such as folic acid/Amoxicillin were synthesized by high energy ball milling and characterized by X-ray diffraction, Transmission electron microscope, zeta potential, dynamic light scattering, Fourier Transform Infra red (FT-IR) measurements, and thermo gravimetric analysis (TGA). FT-IR and TGA measurements show good adsorption of drugs on oleic acid coated nanoparticles. Magnetic measurements indicate that saturation magnetization is larger for amoxicillin coated particles compared to folic acid coated particles. The biocompatibility of the magnetic nanoparticles prepared was evaluated by *in vitro* cytotoxicity assay using L929 cells as model cells. © *2014 AIP Publishing LLC*.
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INTRODUCTION

Investigations on magnetic nanoparticles are currently a topic of extensive research due to the interesting properties arising from their nanoscale dimensions that result in strongly modified magnetic properties when compared to the corresponding bulk materials. Among the magnetic materials, magnetite and heamatite have been extensively studied in their reduced state[1–3] due to their potential for biomedical applications and ease of synthesis. Due to the chemical instability of metallic magnetic nanoparticles, iron as well as other metals such as cobalt, gold, and nickel have been somewhat neglected in favor of its own oxides. Among metallic magnetic nanoparticles, iron nanoparticles are attractive as they have relatively high magnetization and are able to maintain superparamagnetism at larger particle sizes compared to their oxide counterparts.[4] Though iron nanoparticles have received significant attention due to their potential applications in environmental remediation,[5,6] a very few reports are available on biocompatibility of Fe nanoparticles for biomedical applications such as hyperthermia and drug delivery.[7,8] Folic acid, an essential nutrient which also has the ability to preferentially target cancer cells,[9] and amoxicillin, a well known antibiotic, were chosen for coating on iron nanoparticles. In this paper, we report for the first time to the best of our knowledge, the synthesis of iron nanoparticles coated with folic acid/amoxicillin by high energy ball milling and the investigation of their magnetic properties and biocompatibility.

EXPERIMENTAL DETAILS

In order to obtain iron nanoparticles, the iron powder (LOBA Chemie, Electrolytic grade 99.5% purity) was subjected to high energy ball milling using a Fritsch Pulverisette P5 Planetary Mill. The sample was ground in a tungsten carbide vial with 10 mm tungsten carbide balls in the weight ratio of 15:1 with 300 rpm speed in a wet medium using toluene. About 12 wt. % oleic acid (OA) was added to the wet medium and milled for 30 h. Then, folic acid (FA)/amoxicillin (AMX) were taken (75 wt. % of oleic acid) and milled with the OA coated nanoparticles for 3 h at a speed of 50 rpm. The iron nanoparticles coated by oleic acid and drugs have been characterized by X-ray diffraction (XRD), Fourier transform infra red (FT-IR), and thermo gravimetric analysis (TGA). The microstructure of Fe nanoparticles was investigated by transmission electron microscope (TEM). The hydrodynamic diameter, the size distribution, and zeta potential of the nanoparticles were evaluated by dynamic light scattering (DLS) technique by using Zetatrac (Microtrac, Inc., USA) instrument. The magnetic hysteresis loops were recorded on the samples at room temperature by vibrating sample magnetometer (VSM) up to a maximum applied field of 20 kOe.

The *in vitro* cytotoxicity of iron nanoparticles coated with drugs was evaluated on L929 cell lines by using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay as described in the literature.[9] To determine cell cytotoxicity/viability, L929 cells were seeded into 96-well tissue culture plates at a density of 1×10^4 cells/well at 37 °C in a 5% CO_2 atmosphere. After 24 h, the Dulbecco's Modified Eagle medium (DMEM) in the wells was replaced with fresh DMEM containing iron nanoparticles coated with drugs at different concentrations (50, 100, 200, and 500 μg/ml). After 24 h, the medium containing iron nanoparticles coated with drugs was removed, and the cells were washed three times with Phosphate Buffered Saline (PBS). 20 μl of MTT dye solution (5 mg/ml in PBS, pH 7.4) was then added to each well. After incubation for 4 h at 37 °C, the DMEM medium was removed.[10] The formazan crystals formed inside the cells were dissolved with 200 μl of dimethylsulfoxide (DMSO). After 10 min, absorbance was measured spectrophotometrically at 595 nm with a Spectramax

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Plus384® Spectrophotometer (Molecular Devices, CA, USA). The production of formazan is directly proportional to the number of viable cells.[11,12]

RESULTS AND DISCUSSION

The XRD patterns of Fe nanoparticles obtained at zero hour and 30 h milling with OA and FA/AMX are shown in Figure 1. Normally, iron particles at nanoscale size are easy to be oxidized in air. However, XRD pattern could be indexed to cubic structure of iron (JCPDS card, no. 06-0696) with no traces of iron oxide phase detected, which might be due to oleic acid coating on the surface of nanoparticles. The average crystallite sizes estimated using the Scherrer's formula for Fe nanoparticles coated with OA (FeOA), Fe nanoparticles coated with OA and FA (FeOAFA), and Fe nanoparticles coated with OA and AMX (FeOAAMX) are 13, 24, and 24 nm, respectively. TEM microstructure of FeOA sample shows that they have nearly narrow size distribution with size ~11 nm as seen in Figure 2 with no agglomeration. The DLS measurements of FeOA, FeOAFA, and FeOAAMX with particle concentration of 0.02/l in ethanol are shown in Figures 3(a)–3(c), respectively. The average hydrodynamic diameters for FeOA, FeOAFA, and FeOAAMX are 24.64 nm, 319 nm, and 330 nm, respectively. The significantly large hydrodynamic sizes of FeOAFA and FeOAAMX nanoparticles possibly could be due to bond formation between drugs coated on nanoparticles with its adjacent surfaces, which can cause crosslinking between particles and result in a large hydrodynamic size. It is generally accepted that the particles are electrostatically stable if the zeta potential has an absolute value higher than 30 mV.[13]

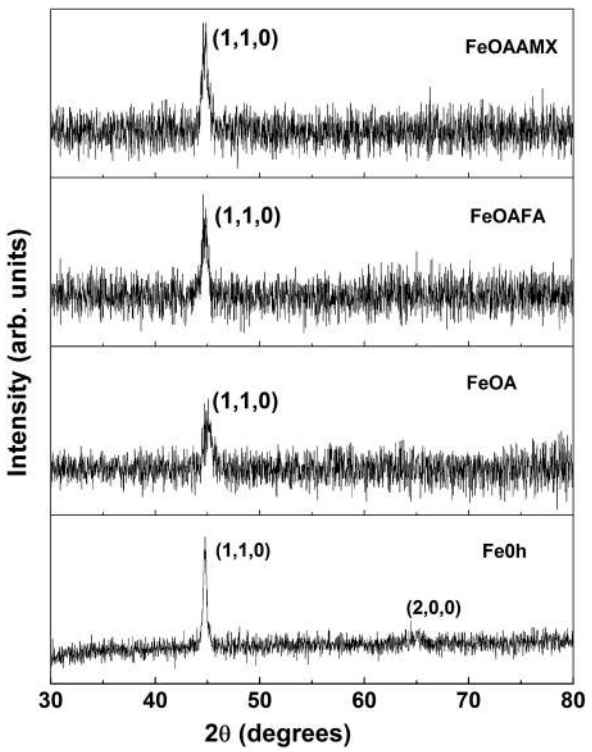

FIG. 1. XRD patterns of Fe nanoparticles obtained at 0 h and 30 h milling with oleic acid and coated with folic acid/amoxicillin.

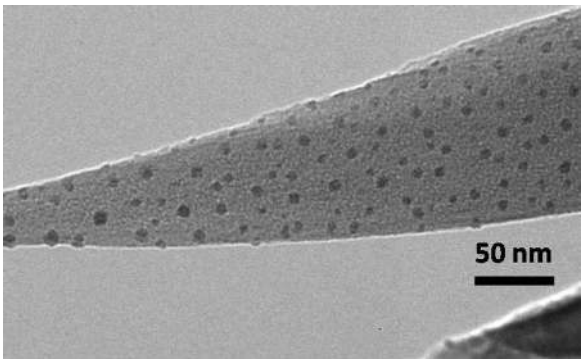

FIG. 2. TEM micrograph of iron nanoparticles coated with oleic acid (FeOA).

The zeta potential value can be used as an indicator of the stability of a colloidal system. The zeta potential for FeOA, FeOAFA, and FeOAAMX is +68 mV, +122 mV, and +129 mV, respectively.

FT-IR spectra of OA,[14] FA,[15] AMX,[16] FeOA, FeOAFA, and FeOAAMX are shown in Figure 4 (The inset shows expanded region FT-IR spectra of the samples between 1000 and 3000 cm^{-1}). In FT-IR spectra of FeOA sample, the

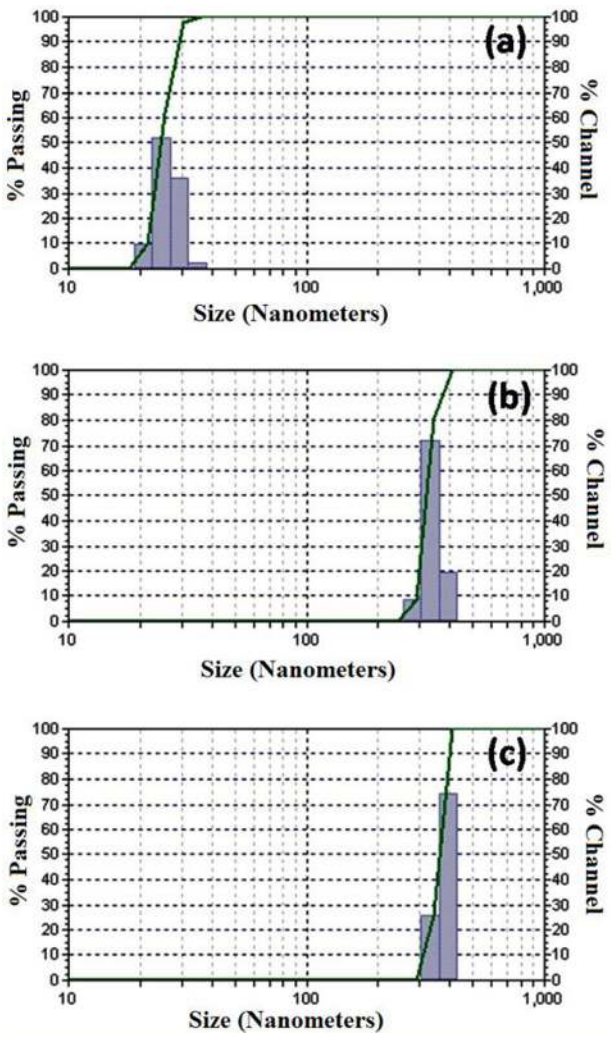

FIG. 3. Particle size distribution plots for (a) FeOA (b) FeOAFA, and (c) FeOAAMX by DLS method.

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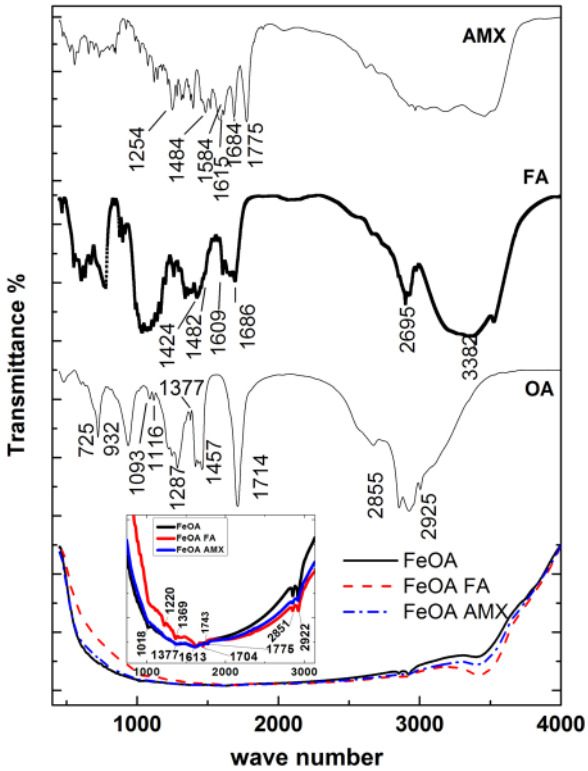

FIG. 4. FT-IR spectra of OA, FA, AMX, FeOA, FeOAFA, and FeOAAMX. The inset shows the expanded region of FT-IR spectra of FeOA, FeOAFA, and FeOAAMX between 1000 and 3000 cm^{-1}.

asymmetric CH_2 stretch and the symmetric CH_2 shifted from 2925 and 2855 to 2922 and 2851 cm^{-1}, respectively. The band from 1710 cm^{-1} (C=O bond asymmetric vibration) has been shifted to 1743 cm^{-1}. A new band can be observed at 1018 cm^{-1}. These results revealed the adsorption of oleic acid on iron nanoparticles. The FT-IR spectra of FeOAFA/FeOAAMX show characteristic bands of both OA and FA/AMX with slight modifications such as shifting of bands, new bands, or missing of some bands due to the chemical bonds occurring between Fe nanoparticles, OA, and FA/AMX. These bands appeared with reduced intensity due to the smaller concentrations. The FT-IR results showed that FA/AMX is adsorbed on the oleic acid coated Fe nanoparticles.

TGA curves of FeOA are characterized by weight losses in two distinct steps in the temperature ranges room temperature to 500 °C and 500–700 °C as shown in Figure 5. Since the total weight loss occurred is matching with the amount of OA added, we ascribe these two steps of weight losses to the secondary and primary OA-coated layer in FeOA nanoparticles, respectively.[17] This confirms that OA is coated on Fe nanoparticles. In the case of FeOAFA sample, the initial weight loss is due to the moisture, and the weight losses at 212, 339, 449, and 597 °C could be due to the decomposition of FA and OA. The decomposition temperatures are slightly altered for both of them. In the case of FeOAAMX sample, the weight losses at temperatures around 125, 222, 356, 421, 554, and 667 °C are due to the decomposition of OA and AMX.[17,18] The decomposition was not complete even at 700 °C. From the TGA curves, it is confirmed that FA and

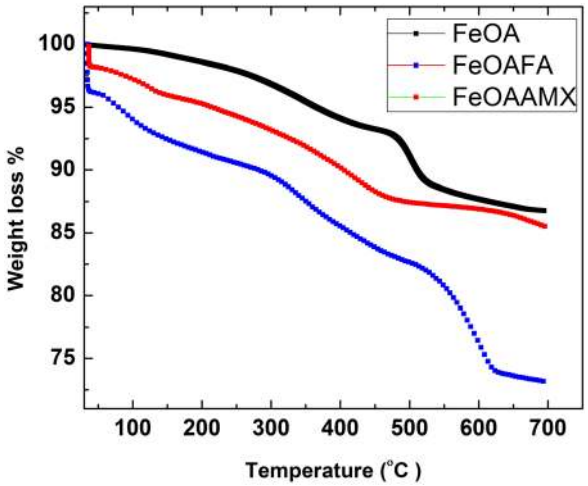

FIG. 5. TGA measurements of FeOA, FeOAFA, and FeOAAMX.

AMX have been adsorbed on Fe nanoparticles coated with OA.

The magnetic hysteresis loops measured at room temperature on FeOA, FeOAFA, and FeOAAMX are shown in Figure 6. The saturation magnetization (M_s) values for FeOA, FeOAFA, and FeOAAMX are 172.3, 144.5, and 149 emu/g per Fe, respectively. The decrease in M_s values compared to that of bulk Fe ($M_s = 212$ emu/g) could be due to the reduced size and the coatings covered around them. In the case of FeOAFA, the M_s value is slightly less than that of FeOAAMX though their particle sizes are same, which may be due to the more carbon chain involved in FA coating compared to that of AMX. The fact that the samples having coercive field ~100 Oe indicates that they have hysteresis losses suitable for heat induced biomedical applications like hyperthermia, etc.[19]

The biocompatibility of the samples is shown in Figure 7, and the cell viability is inversely proportional to the concentration of the coated nanoparticles used as seen in Fig. 6. At 50 μg/ml, the iron nanoparticles coated with FA and AMX have shown 91.49 and 90.05% cell viability, respectively. The cell viability of FeOAAMX is more

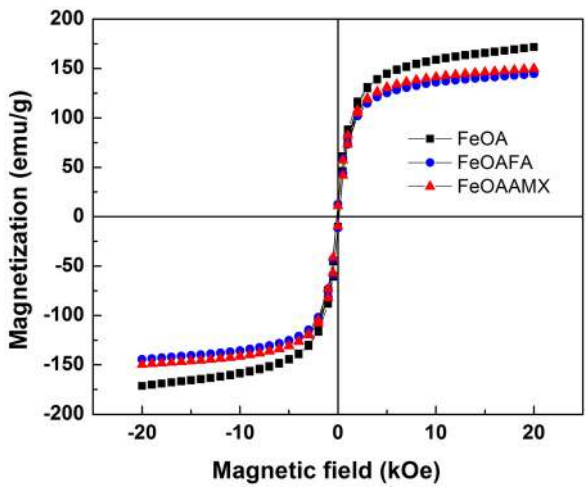

FIG. 6. Magnetic hysteresis loops of FeOA, FeOAFA, and FeOAAMX.

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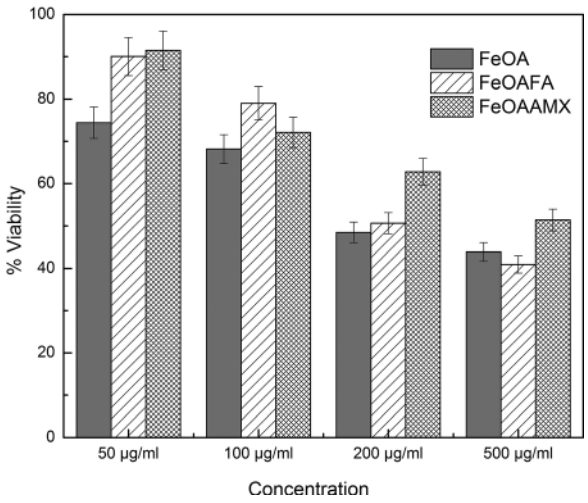

FIG. 7. MTT assays on L929 cell lines for different concentrations of FeOA, FeOAFA, and FeOAAMX nanoparticles.

compared to that of FeOA and FeOAFA at all the concentrations done (within the error bar limits), indicating its more biocompatibility.

SUMMARY AND CONCLUSIONS

The iron nanoparticles were synthesized by high energy ball milling coated by OA, and we have first reported to the best of our knowledge that drugs like FA/AMX can also be coated on the magnetic nanoparticles by ball milling apart from chemical methods. The biocompatibility of the magnetic nanoparticles prepared was evaluated by *in vitro* cytotoxicity assay. Having high M_s values, hysteresis loss and biocompatibility make iron nanoparticles more suitable for *in vivo* biomedical applications such as biosensing, hyperthermia, etc.

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