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Water dispersible oleic acid-coated Fe_3O_4 nanoparticles for biomedical applications

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A B S T R A C T

Fe_3O_4 magnetic nanoparticles (MNPs) have proved their tremendous potential to be used for various biomedical applications. Oleic acid (OA) is widely used in ferrite nanoparticle synthesis because it can form a dense protective monolayer, thereby producing highly uniform and monodispersed particles. Capping agents such as oleic acid are often used because they form a protective monolayer, which is strongly bonded to the surface of nanoparticles. This is necessary for making monodisperse and highly uniform MNPs. Coating of Fe_3O_4 MNPs with OA makes the particles dispersible only in organic solvents and consequently limits their use for biomedical applications. Hence, in this work, the OA coated MNPs were again functionalized with chitosan (CS), in order to impart hydrophilicity on their surface. All the morphological, magnetic, colloidal and cytotoxic characteristics of the resulting core–shells were studied thoroughly. Their heating induction ability was studied to predict their possible use in hyperthermia therapy of cancer. Specific absorption rate was found to be increased than that of bare MNPs.

1. Introduction

Nanosized magnetic iron oxide nanoparticles such as magnetite coated with hydrophilic water soluble polymers have received increasing attention due to their wide ranging biomedical applications in drug delivery [1], resonance imaging (MRI) contrast agents [2], in high-gradient magnetic field separations [3], treatment of retinal detachment [4], in bio-catalysis [5], as magnetic bio-separations [6] *etc.* Owing to their biocompatibility and low toxicity, magnetite (Fe_3O_4) nanoparticles (NPs) are the most attractive candidates for use in human body. Magnetic NPs (MNPs) for biomedical applications should fulfill a variety of requirements, including: (i) superparamagnetic behavior at room temperature, in order to avoid particle aggregation; (ii) large saturation magnetization, so as to show a large response under the application of a magnetic field; (iii) a limiting size in the order of 20 nm for *in vivo* applications, and (iv) bio-compatibility, such that nanoparticles are usually coated with either biological or biocompatible molecules.

In order to make MNPs suitable for *in vivo* applications, they are coated with various polymers like chitosan (CS) [7], oleic acid (OA) [8], starch [9], pullulan [10], dextran [11] *etc.* Out of these, OA is probably the most common small molecule which is complexed with magnetite [12]. OA possesses a non-polar hydrocarbon tail and a polar carboxylic acid head group. Carboxylate anions are known to coordinate with the surface of magnetite, presumably through a coordination of iron atoms with both the carboxylate oxygens. The polar head group anchored on the magnetite surface, the non-polar tail extends into solution, causing the magnetite to be hydrophobic and dispersible in organic solvents.

However coating of MNPs with OA makes the particles only dispersible in organic solvents and this limits their use for medical applications [13]. Fe_3O_4 NPs modified with OA decrease degree of agglomeration and favor the generation of hydrogen bonding between Fe_3O_4 and CS, thus improve their interfacial combination. Considering biodegradability and toxicity, much attention have been paid to chitosan, since it possesses interesting properties such as biocompatibility, biodegradability, film forming ability, gelation characteristics and bioadhesion. Another advantage of CS is that the amine groups can offer a variety of active sites for further biofunctionalization [14].

In the present investigation, Fe_3O_4 nanoparticles were prepared by alkaline precipitation method and were surface modified with OA. The precursor used for the synthesis of Fe_3O_4 was ferrous chloride only. A very little literature is available on the synthesis of Fe_3O_4 using $FeCl_2$ only as the sole precursor for the nanoparticles [15,16]. The as-formed NPs were used for surface modification using OA which is hydrophobic in nature. Hence in order to make it suitable for biomedical applications, OA-Fe_3O_4 MNPs were further coated with CS, a biopolymer having hydrophilic nature. Bare (Fe_3O_4), OA coated (OA-Fe_3O_4) and CS-coated OA-Fe_3O_4 (CS-OA-Fe_3O_4) nanoparticles were characterized further for their

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structural, morphological and magnetic properties in order to investigate the role of coating on particle. The resulting particles were well dispersed in water and studied for their heating induction effect so as to study their possible use in hyperthermia application.

2. Materials and method

2.1. Materials

Ferrous chloride ($FeCl_2 \cdot 4H_2O$), HCl, acetic acid, acetone and NaOH were procured from HiMedia, Mumbai (Maharashtra), India. Methanol, chitosan and oleic acid were purchased from Sigma-Aldrich, USA. Double distilled water was used throughout the procedure.

2.2. 2 Experimental

2.2.1. Synthesis of Fe_3O_4 nanoparticles

Fe_3O_4 nanoparticles were synthesized *via* alkaline precipitation [17,18]. The brief procedure for preparation of MNPs is as follows:

2 g $FeCl_2 \cdot 4H_2O$ was dissolved in 50 mL 1 M HCl by heating upto 70 °C. 50 mL 3 M NaOH was added to it at 60 °C drop by drop with constant stirring. A black precipitate gets formed. This is nothing but magnetite (Fe_3O_4) NPs. The possible reaction taking place is showed below:

$$3\ FeCl_2{\cdot}4H_2O + 6\ NaOH + ½\ O_2 \rightarrow Fe_3O_4 + 6\ NaCl + 15\ H_2O \tag{1}$$

The precipitate is allowed to settle down. The rate of settling is increased by applying external magnetic field. The precipitate is then separated, washed with D/W till the neutral pH, dried at RT and used further studies. The particles studied for various characters using XRD, SEM, TEM, FTIR, VSM and temperature susceptibility.

2.2.2. Surface modification of Fe_3O_4 nanoparticles with OA

As-formed Fe_3O_4 MNPs were used for coating procedure. The particles were well dispersed in 200 ml methanol by ultrasonication. 50 ml OA was added while constant stirring at 80 °C. OA-Fe_3O_4 particles were filtered through Whatman filter paper no.1, washed three times with distilled water. The OA-Fe_3O_4 particles were separated from the filter paper using acetone. The particles were dried at room temperature to evaporate all the acetone. These particles were termed as OA-Fe_3O_4 NPs.

2.2.3. Coating of CS on OA-Fe_3O_4 NPs

As-formed OA-Fe_3O_4 MNPs were used for coating procedure. The were dispersed in 1% CS solution in 2% acetic acid. The mixture was ultrasonicated for 30 min. After ultrasonication, the CS-coated Fe_3O_4 NPs (CS-OA-Fe_3O_4) allowed to settle, washed with distilled water 3 times to remove excess CS, separated and dried at 50 °C. These OA-particles were also thoroughly studied for their structural, morphological and magnetic characters. The coating of CS on Fe_3O_4 MNPs was confirmed by FTIR spectra and TG-DTA results.

2.3. Characterizations

Bare, OA-Fe_3O_4 and CS-OA-Fe_3O_4 MNPs were studied for their structural, morphological and magnetic characters using XRD, FT-IR, EDAX, SEM, TEM, VSM and tTemperature susceptibility. In order to use the particles for hyperthermia therapy, they were studied for their heating induction ability.

The XRD pattern of MNPs drop coated and air-dried on the glass substrate was recorded to study the structural and phase analysis by XRD Philips PW-3710 diffractometer using CuK$_\alpha$ radiation in the 2θ range from 0 to 100°. The XRD patterns were evaluated by X′pert high score software and compared with JCPDS card. The average crystallite size (t) was calculated from the diffraction line-width of XRD pattern, based on Scherrer′s relation:

$$t = 0.9\lambda/\beta \cos\theta \tag{2}$$

Where, β is the full width at half maximum (fwhm).

TEM images were used to determine the morphology and size of the MNPs. For this purpose, the colloidal solution of the MNPs was transferred on to a carbon coated copper grid and allowed to air dry. The grid was then scanned using Philips CM200 model Transmission electron Microscope, operating voltage 20–200 kV with resolution 2.4 Å. The compositional analysis was done by Energy-dispersive analysis of X-ray spectroscopy (EDAX, JEOL JSM 6360). The Perkin-Elmer spectrometer, (Model no.783, USA) was used to get FT-IR spectra of MNPs in the range from 450 to 4000 cm^{-1} using KBr pellets to check the possible interaction of OA-Fe_3O_4 with chitosan.

The magnetization measurements were performed on a superconducting quantum interference device (SQUID) magnetometer to investigate the saturation magnetization (Ms), blocking temperature (T_B) and Curie temperature (T_C). The measurements include field dependent hysteresis loops, (M–H), at two different temperatures 100 and 300 K with applied field range from 0 to $\pm 1 \times 10^3$ Oe.

Induction heating of Fe_3O_4 nanoparticles for hyperthermia application was performed in a plastic micro centrifuge tube (1.5 ml) using an induction heating unit (Easy Heat 8310, Ambrell; UK) with a 6 cm diameter (4 turns) heating coil. To keep the temperature of the coil at ambient temperature, a provision of water circulation in coils was provided. MNPs suspended in 1 ml of distilled water was placed at the center of the coil and the applied frequency was 265 kHz. Particles are dispersed in water with a concentration ranging from 2, 5 and 10 mg ml^{-1} and ultrasonicated for 20 min to achieve a good dispersion of the NPs in carrier fluid. Samples were heated for 10 min with the desired current (200–400 A). For the conducted experiments, the magnetic field was calculated from the relationship:

$$H = \frac{1.257ni}{L}\ \textit{in Oe} \tag{3}$$

where, n, i and L denote the number of turns, applied current and the diameter of the turn in centimeters, respectively. Calculated values of the magnetic field (H) at 200, 300 and 400 A were 167.6, 251.4 and 335.2 Oe (equivalent to 13.3, 20.0 and 26.7 kA m^{-1}), respectively. Temperature was measured using an optical fiber probe with accuracy 0.1 °C.

3. Results and discussions

Fig. 1(a) and (b) shows the powder XRD patterns for bare and OA-CS-coated Fe_3O_4 NPs respectively. The main characteristic peaks were obtained with the (hkl) values of (220), (311), (400), (422) and (511). These were then matched with the JCPDS file number 82-1533, which corresponds to Fe_3O_4 phase. Both the NPs show inverse spinel structure. The crystallite sizes of NPs were calculated from FWHM of the most intense peaks using the Debye–Scherrer formula. The crystallite sizes obtained were 20 nm and 10 nm for bare and OA-CS-coated MNPs respectively. However, the peaks became broad and intensity decreased after capping of Fe_3O_4 with OA-CS and hence it can be clearly stated that the particle size decreased after coating procedure. The coating of amorphous OA-CS polymer on crystalline Fe_3O_4 may induce

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