Applied Surface Science 428 (2018) 492–499

Contents lists available at ScienceDirect

Applied Surface Science

journal homepage: www.elsevier.com/locate/apsusc

Full Length Article

Effects of rare earth doping on multi-core iron oxide nanoparticles properties

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ARTICLE INFO

Article history:
Received 17 July 2017
Accepted 19 September 2017
Available online 20 September 2017

Keywords:
Multicore iron oxide nanoparticle
Protein adsorption
Rare earth metals
Nanomagnetism
Doping

ABSTRACT

New multi-core iron oxide magnetic nanoparticles doped with rare earth metals (Gd, Eu) were obtained by a one step synthesis procedure using a solvothermal method for potential biomedical applications. The obtained clusters were characterized by X-ray diffraction (XRD), transmission electron microscopy (TEM), energy-dispersive X-ray microanalysis (EDX), X-ray photoelectron spectroscopy (XPS) and magnetization measurements. They possess high colloidal stability, a saturation magnetization of up to 52 emu/g, and nearly spherical shape. The presence of rare earth ions in the obtained samples was confirmed by EDX and XPS. XRD analysis proved the homogeneous distribution of the trivalent rare earth ions in the inverse-spinel structure of magnetite and the increase of crystal strain upon doping the samples. XPS study reveals the valence state and the cation distribution on the octahedral and tetrahedral sites of the analysed samples. The observed shift of the XPS valence band spectra maximum in the direction of higher binding energies after rare earth doping, as well as theoretical valence band calculations prove the presence of Gd and Eu ions in octahedral sites. The blood protein adsorption ability of the obtained samples surface, the most important factor of the interaction between biomaterials and body fluids, was assessed by interaction with bovine serum albumin (BSA). The rare earth doped clusters surface show higher afinity for binding BSA. In vitro cytotoxicity test results for the studied samples showed no cytotoxicity in low and medium doses, establishing a potential perspective for rare earth doped MNC to facilitate multiple therapies in a single formulation for cancer theranostics.

1. Introduction

Magnetic iron oxide nanoparticles with a large variety of functional coatings show great promise for innovative applications in nanomedicine such as nanotherapeutics, multimodal imaging, targeted drug delivery, hyperthermia, analyte monitoring, or enzyme, protein and nucleic acid separations [1–6]. Their characteristics such as facile and non-toxic cellular up-take, superparamagnetic behaviour, a high saturation magnetization/large magnetic moment of particles, a good response to moderate magnetic fields, their inherent ability to cross biological barriers, provision of a large surface area for conjugating targeting ligands motivate the increasing interest and the intensive research efforts for improving the required properties for biomedical applications. The existing magnetic nanomaterials still have shortcomings (toxicity, aggregation, irreversible interaction with blood components, incomplete drug delivery), therefore the design and development of optimal magnetic carriers for medical applications remains a challenge for future research. Among magnetic carriers, functionalized magnetic nanoparticles cluster (MNC) represent a novel type of carriers with great potential for controlled and targeted therapy and diagnosis [5–7]. The main advantage of controlled clusterization of magnetic nanoparticles into multi-core nanosystems arises from their high magnetic moment in an external magnetic field which results in superior contrast ability for magnetic resonance imaging (MRI) and efficient manipulation/targeting as well as magnetic separation [8–11]. The magnetic properties of nanoparticles are strongly influenced by their size distribution, chemical composition and magnetic interactions [12,13]. It is worth to note that, at the nanoscale level, a reduction in the particle size often implies a decrease in the saturation magnetization due to the surface spin-canting effect [14,15]. In this context, the magnetite nanoparticle clusters represent promising systems with high saturation mag-

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http://dx.doi.org/10.1016/j.apsusc.2017.09.160
0169-4332/

netization, size control and controlled magnetic properties by rare earth doping. Recent studies show that once dispersed in biological fluids the nanoparticles are rapidly coated with proteins and as a consequence formation of a protein corona occurs. This phenomena is due to both, the high frequency of collisions between the proteins and nanoparticles and the high binding affinity of the proteins. A high-affinity association with proteins is undesirable, as it masks the targeting or molecular recognition properties of the nanoparticles. Therefore, the nature of the magnetic particles surface coating should ensure biocompatibility and stability while preventing the nonspecific adhesion of plasma proteins on the particles surface. Thus, for application of nanocariers in nanomedicine advanced coatings are required in order to reduce protein adsorption from physiological fluids while increasing their in vivo biodistribution (stealth effect). Among multiple strategies and efforts to enrich the stealth effect of nanocariers, it was published recently that a simple way to enrich the **"stealth effect"** of nanocarriers is by mixing them with human plasma [16]. In this context, here we have designed and prepared by a solvothermal polyol process method [17] multi-core iron oxide nanoparticles doped with rare earth elements (Eu^{3+} and Gd^{3+}) self assembled by EDTA for biomedical applications. Even though EDTA is known as a very good complexing agent [18,19], here we showed that it has good properties for organizing iron oxide nanoparticles in spherical clusters with high colloidal stability as well. To study the effect of rare earth doping on the structure and properties of magnetite nanoparticles clusters the morphology, structure, crystallite size,elemental composition, electronic structure and magnetic properties of the obtained samples were determined by transmission electron microscopy (TEM), energy dispersive X-ray analysis (EDX), X-ray diffraction (XRD), X-ray photoelectron spectroscopy (XPS) and magnetization measurements. Testing protein interaction with MNC surface before and after rare earth doping is very important in order to further develop the next generation of biomaterials with bioinert/biospecific surfaces. To investigate the way EDTA and rare earth doping influence protein adsorption in terms of interaction of magnetic clusters with proteins bovine serum albumin (BSA) was chosen as a model protein, for its structural similarity with human serum albumin [20]. Regarding the plasma proteins content, serum albumin is the most abundant protein accounting for 60–70% from the measured proteins being the first entities which come into contact with a foreign object from the blood. Protein adsorbtion on the sample surface was analysed by UV–vis and Fourier transform infrared (FTIR) spectroscopy. To evaluate sample toxicity cell viability results are also presented on HaCaT cell cultures.

2. Experimental section

2.1. Materials and synthesis method

Iron(III) chloride hexahydrate ($FeCl_3{\cdot}6H_2O$), gadolinium hexahydrate ($GdCl_3{\cdot}6H_2O$), europium hexahydrate ($EuCl_3{\cdot}6H_2O$), sodium acetate anhydrous (CH_3-COONa, NaAc), and ethylenediaminetetraacetic acid disodium salt (EDTA) were purchased from Sigma-Aldrich. Ethylene glycol (EG) and diethylene glycol (DEG) were provided by Alfa Aesar. All chemicals were of analytical grade and used without further purification. The magnetite nanoparticles clusters (MNC) undoped and doped with rare earth ions Gd and Eu were obtained by solvothermal method. $FeCl_3{\cdot}6H_2O$ (95, 90, 85% molar) with different molar concentrations of REs (5, 10 15% molar), Table 1, and NaAc (36 mmol, 3 g) were dissolved in a 1:1 mixture of EG and DEG (40 ml) to form a clear solution under mechanical stirring at 60 °C for 3 h.

The synthesis parameters (metal salts quantities) for magnetite nanoclusters doped with Gd and Eu are shown in Table 1. Separately, EDTA (0.89 mmol, 0.3 g) were suspended in a 1:1 mixture of DEG and EG (20 ml) at room temperature for 3 h. The two solutions were mixed together in a Teflon-lined stainless steel autoclave (80 ml volume), sealed and heated at 200 °C. After 14 h reaction time, the autoclave was cooled to room temperature. The resulting dark suspension was magnetically separated and washed several times with methanol water mixture and distilled water. The magnetic clusters were kept in 10 ml water as a suspension.

Table 1
Synthesis parameters of the MNC doped with RE.

Sample	Metal salts quantities (mmol and grams)		
	$FeCl_3{\cdot}6H_2O$	$GdCl_3{\cdot}6H_2O$	$EuCl_3{\cdot}6H_2O$
MNC Gd-5	7.03 mmol, 1.9 g	0.37 mmol, 0.137 g	–
MNC Eu-5		–	0.37 mmol, 0.135 g
MNC Gd-10	6.66 mmol, 1.8 g	0.74 mmol, 0.274 g	–
MNC Eu-10		–	0.74 mmol, 0.271 g
MNC Gd-15	6.29 mmol, 1.7 g	1.11 mmol, 0.412 g	–

2.2. Characterization methods

Transmission electron microscopy (TEM) and scanning electron microscopy (SEM) was performed by a Hitachi SU 8230 equipped with energy dispersive X-ray analysis (EDX) in order to determine the morphology and elemental composition of the prepared samples. The electron energy used was 30 keV. In order to amplify the secondary electron signal, the samples prepared in the form of powders were metalized with an Au thin layer of 10 nm in an automatic Sputter Coater, in argon atmosphere. The surface chemical composition of the samples was investigated by X-ray Photoelectron Spectroscopy (XPS) using a spectrometer SPECS equipped with an Al/Mg dual-anode X-ray source, a PHOIBOS 150 2D CCD hemispherical energy analyzer, and a multichanneltron detector with vacuum maintained at 1×10^{-9} Torr. The Al K_α X-ray source (1486.6 eV) was operated at 200 W. The XPS survey spectra were recorded at 30 eV pass energy and 0.5 eV/step. The high-resolution spectra for the individual elements (Fe, Gd, Eu) were recorded by accumulating 10 scans at 30 eV pass energy and 0.1 eV/step. Data analysis and deconvolutions was performed using CasaXPS software with a Gaussian-Lorentzian product function and a nonlinear Shirley background subtraction. Peak shifts due to any apparent charging were normalized with the C 1 s peak set to 284.8 eV. X-ray diffraction (XRD) measurements were performed using a Shimadzu 6000 XRD difractometer with Cr Kα radiation, source power of 40 kV and 30 mA at room temperature. Magnetic characterization of the samples at room temperature was performed using a Vibrating Sample Magnetometer Cryogenics. FTIR spectra were carried out on a JASCO FTIR 610 spectrophotometer in transmission configuration in the range 4000–400 cm^{-1} and spectral resolution 4 cm^{-1} using KBr pellet technique. UV–vis spectroscopy measurements were performed with a Jasco V550 UV–vis spectrometer with the spectral resolution of 2 nm in the 190–900 nm wavelengths range using 10 mm length quartz cells.

2.3. Protein adsorption

To assay the protein adsorbtion on the sample surfaces by FTIR and UV VIS techniques, Bovine Serum Albumin (BSA) (Sigma Aldrich with molar mass 69 kDa) solution was prepared in phosphate buffer solution (PBS) with pH7.4. Powdered samples were immersed in BSA solution (0.7 mg/mL) and subsequently placed at 37 °C for