

SYNTHESIS AND CHARACTERIZATION OF PEG-SILANE FUNCTIONALIZED IRON OXIDE (II, III) NANOPARTICLE AS POTENTIAL MRI CONTRAST AGENT

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Abstract

Herein we report the synthesis, functionalization and characterization of superparamagnetic and ferrimagnetic iron oxide (II, III) nanoparticles with different shape and size. Using a number of chemical methods magnetite nanoparticles having a spherical shape and size: 9 nm (coprecipitation), 22 nm and 50 nm (redox reaction), and 40 nm cubes were synthesized. Special attention in this paper is devoted to covalent modification of magnetite nanoparticles by polymers, such as silane-polyethylene glycol (Peg-silane). The major advantage of modified by polymer magnetic nanoparticles is low toxicity, colloidal stability of prepared magnetite nanoparticles and possibility for post functionalization. We determined coercivity and saturated magnetism. Also, the relaxivity T₂ was measured by magnetic resonance imaging (MRI). Prepared nanoparticles have great interest and potential for use in biomedical imaging.

Keywords: Magnetic nanoparticles, polymer coating, MRI agents, functionalization of nanoparticles

1. INTRODUCTION

Nanomaterials have attracted interest during the last decade as they exhibit unique properties compared with corresponding bulk materials [1-3]. Nanomaterials based on metal oxides are widely used in different fields of science and technology [4]. Magnetic iron oxide nanoparticles with functionalized surface have shown great potential applications in biomedicine and pharmacology, including magnetic resonance imaging (MRI) contrast enhancement [5], targeted drug delivery [6], hyperthermia [7], etc. All these applications require that these nanoparticles have been biocompatible and nontoxic. Also, magnetic nanometer-scaled materials, generally smaller than 100 nm, exhibit very different magnetic properties [8-10]. Nowadays, many studies highlight the multimodal imaging and the last advances in theranostic which combines diagnosis and therapy. Magnetite nanoparticles synthesis can be carried out by several synthetic approaches in organic [11] or water solutions [12], but all of them have advantages and disadvantages. In this work we used various procedures for optimal synthesis of magnetic nanoparticles with different size and narrow size distribution. The main problem with water solution of magnetite nanoparticles is their colloidal stability. To solve this problem we made the covalent surface modification of obtained particles through the coating with 3-aminopropyltriethoxysilane (APTES) coupled with a polyethylene glycol (PEG).

2. EXPERIMENTAL SECTION

2.1. Measurements

TEM: Transmission electron microscope JEOL JEM-2100F/Cs/GIF (200 kV, 0.8 A)

DLS: Zetasizer Nano ZS (173°) with He-Ne laser (633 nm, 5 mV)

XRD: RigakuUltima IV diffractometer using CoK α - radiation and graphite monochromator. Processing of the spectra was carried out using the Rietveld method implemented in the software product PDXL (Rigaku).

Magnetic properties: To determine the magnetic properties of the hysteresis loop was built on vibromagnetometre VSM 250 in the range from -20 kOe to 20 kOe

T2 relaxivity: ClinScan 7T (70/30) MRI system (Bruker Biospin, USA). Tubes containing MNPs solutions with different iron concentrations (0,4; 0,2; 0,1; 0,05; 0,01 mmol) were imaged by MRI using Multi Spin Echo (MSE) technique with TR=10000ms and TE= 8, 16, 24,..., 160. Signal intensity (SI) was measured by ImageJ (NIH, USA) software and SI dependence on TE was modeled by the following equation using MathCad (PTC, USA): $SI = S_0 * e^{\left(\frac{-TE}{T_2}\right)}$ For each T2 was calculated according to the formula above. 1/T2 was plotted against corresponding Fe concentration and T2 relaxivity was calculated as a slope of the curve.

2.2. Synthesis of magnetite nanoparticles (9 ± 2 nm)

The iron oxide (magnetite) nanoparticles were synthesized by coprecipitation method [13]. Aqueous solutions of 4 ml of 1 M Iron(III) chloride and 1 ml of 2 M Iron(II) chloride with the addition of 10 ml of 2 M HCl were mixed and diluted by 50 ml of 0.7 M NH₃(28%). The mixture was stirred for 30 min at 500 rpm. Then, the precipitate was separated by magnetic decantation. The precipitate was stirred and diluted with 50 ml of 2 M HClO₄ and was then collected by magnetic decantation. The final residue was brought to 50 ml using water. For magnetic measurements magnetite nanoparticles were collected by magnetic decantation, and diluted with ethanol, prepared suspension was evaporated under reduce pressure.

2.3. Synthesis of magnetite nanoparticles (22 ± 4 nm)

The iron oxide(magnetite) nanoparticles were synthesized by oxidation of iron (II) salt [14]. 25 ml 0,1M aqueous solution of iron (II) chloride was degassed by blowing with argon. pH of the solution was adjusted to 7.8 using based solution (1 M potassium hydroxide). Solution became dark green. Iron salt was oxidized by 0,375 μ l 3% hydrogen peroxide in US-bath. The solid was separated by magnetic decantation and washed several times with distilled water.

2.4. Synthesis of magnetite nanocubes (40 ± 5 nm) and nanospheres (50± 6 nm)

The iron oxide(magnetite) nanoparticles average size 40 and 50 nm were synthesized by oxidation of iron (II) salt by potassium nitrate in alkaline medium [15]. The FeSO₄ precipitation and the subsequent ageing were carried out in a closed system consisting of a three-necked round bottom flask placed in a water bath with mechanical stirring. Argon was flowed and the iron (II) solution was introduced at a constant rate. At first two solutions were bubbled by argon: (1) 180 ml of water containing NaOH and KNO₃ to obtain the final concentration of 7.0 × 10⁻²M NaOH and 0.1M KNO₃ and (2) 20 ml of FeSO₄·7H₂O at different concentrations to obtain final values of 2,5× 10⁻² and 5 × 10⁻²M and dissolved in 10⁻² M H₂SO₄. Using different concentrations allow preparation magnetite nanoparticles with different morphology. Using 2,5× 10⁻² and 5 × 10⁻²M concentrations provides preparation spheres (50 nm) and cubes (40 nm) respectively. After 2 h, the iron (II) sulphate solution was added to the basic solution at a constant rate and under mechanical stirring. When the precipitation was completed, argon was allowed to pass for another 5 min, and the system was undisturbed and heated to 90 °C for 24 h. After cooling the solid was separated by magnetic decantation and washed several times with distilled water.

2.5. Synthesis of N-(4,4,4-Triethoxysilanebutyl)succinamic Acid (1) [16].

1 g (9.9 mmol) of succinic anhydride was dissolved in dry 1,4-dioxane at 60 °C under an argon atmosphere. At room temperature, 2.1 mL (9.0 mmol) of (3-aminopropyl)-triethoxysilane in 10 mL of 1,4-dioxane were added dropwise. The solution was stirred for 1 h and then filtered. After the evaporation of the solvent, a

colorless oil was obtained and characterized. ¹H NMR (ppm) (400 MHz, CDCl₃): δ 0.56–0.64 (t, 2H), 1.15–1.22 (t, 9H), 1.50–1.66 (m, 2 H), 2.44–2.50 (t, 2H), 2.60–2.71 (t, 2H), 3.15–3.25 (m, 2H), 3.77–3.89 (q, 6H), 6.58–6.64 (s, 1H), 10.39–10.88 (s, 1H).

2.6. Synthesis of PEG-silane (2)

Reaction was carried out according to previous work [17].

3. RESULTS AND DISCUSSION

In our study we presented different approaches for the synthesis of magnetite nanoparticles. For the synthesis of magnetite nanoparticles two main methods are used: coprecipitation from iron (II,III) salts and redox reactions using different conditions. We have a goal to synthesize magnetite nanoparticles without shell (organic or polymer), only with hydroxyl groups on the surface. We used two approaches: to synthesize small magnetite nanoparticles with average size 8 nm we used coprecipitation of iron (II, III) chlorides hydrates with ammonia solution. When we prepared bigger nanospheres we used oxidation of iron (II) sulfate by hydrogen peroxide (average size 20 nm) and potassium nitrate (average size 50 nm). Changing the concentration of reagents we can vary morphology of nanoparticles. Using different concentration of iron (II) sulfate in experiment with potassium nitrate apply to prepare nanocubes with average size 40 nm. So, we prepared four different samples with different morphology.

Transmission electronic microscopy image of iron oxide nanoparticles synthesized by coprecipitation was shown in Fig.1A. The average particle size of nanoparticle was 9 nm. The hydrodynamic particle diameter of nonfunctionalized iron oxide dispersed in water was shown in Table 3. Nonfunctionalized particles are aggregated in agglomerates with average size 100-200 nm. This iron oxide nanoparticles can be stabilized by silane-peg covalent coating, which will be demonstrated later.

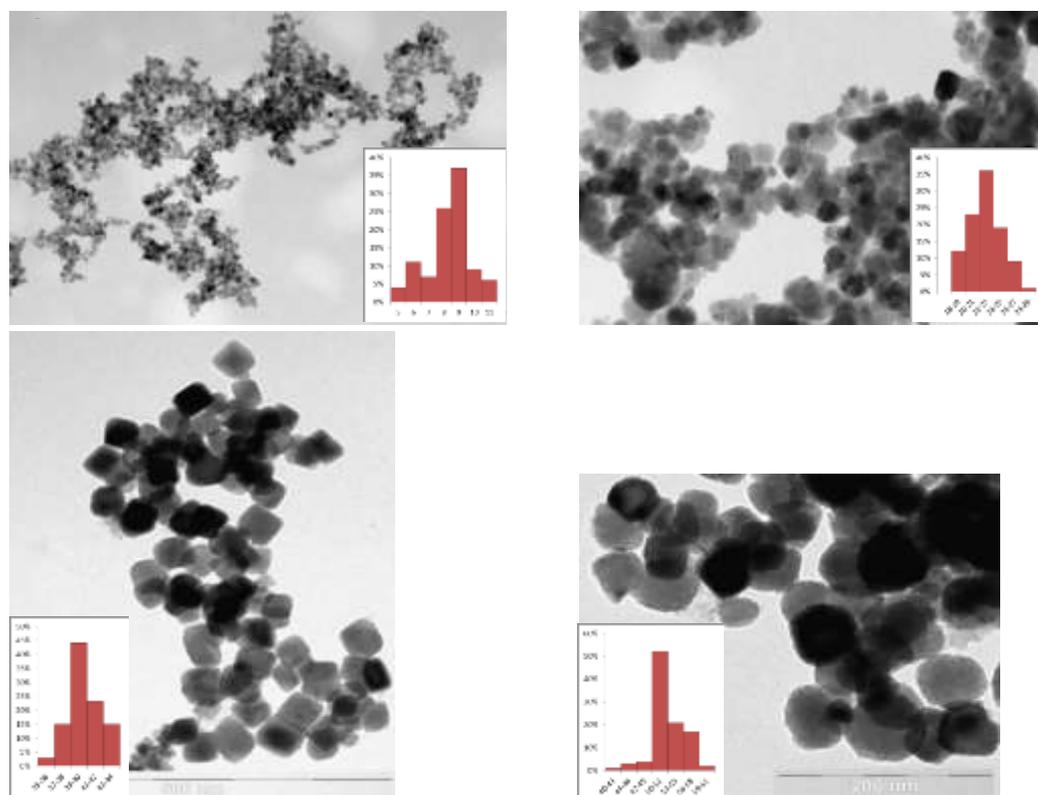


Fig. 1 TEM images of magnetite (A - 9 ± 2 nm, B - 22 ± 4 nm, C - 40 ± 5 nm, D - 50 ± 6 nm)

Nanoparticles with bigger size 22 ± 4 nm were synthesized by oxidation iron (II) chloride under neutral conditions (pH 7,8) by hydrogen peroxide. Transmission electronic microscopy image of iron oxide nanoparticle with average size 22 nm was shown in Fig.1B. Nonfunctionalized particles are aggregated in big agglomerates with average size 800 nm (DLS data) and are not stable in water solution. For larger magnetite nanoparticles we changed oxidant (from hydrogen peroxide to potassium nitrate), and varied ratios of the reactants. The use of a mild oxidant allows to obtain nanoparticles of larger diameter. Also variation of concentrations give change of the morphology. The reaction between iron (II) sulfate and potassium nitrate in alkaline medium gives nanocubes (average size 40 nm) and nanospheres (average size 50 nm) depending on the concentration of iron (II) sulfate: 5×10^{-2} M and $2,5 \times 10^{-2}$ respectively. Transmission electronic microscopy image of iron oxide cubes and spheres was shown in Fig.1C and 1D. Once these conditions were established, the dramatic change in particle size and morphology from spheres to cubes was determined by the iron salt concentration according to a previous work [18] and related to the excess in Fe^{2+} or OH^- in the initial reaction media. Nonfunctionalized particles are aggregated in big agglomerates with average size 250 nm (spheres) and 700 nm (cubes) and are not stable in water solution long time.

Magnetite nanoparticles crystals, prepared by magnetic decantation and evaporation of solvent, were analyzed by XRD powder reflection. Reflex pattern of all magnetite nanoparticles look the same, for example diffraction pattern of magnetite with average size 9 ± 2 nm was shown in Fig. 2 and all data are in table 1. The reflex pattern was compared with the standard reflex pattern of magnetite and shows identical pattern with standard crystal form.

Table 1 Lattice constants of magnetite samples

Nanoparticles	Size (TEM)	Lattice constant, Å
Spheres	9 ± 2 nm	8.374 ± 0.001
Spheres	22 ± 4 nm	8.383 ± 0.001
Cubes	40 ± 5 nm	8.367 ± 0.001
Spheres	50 ± 6 nm	8.385 ± 0.001

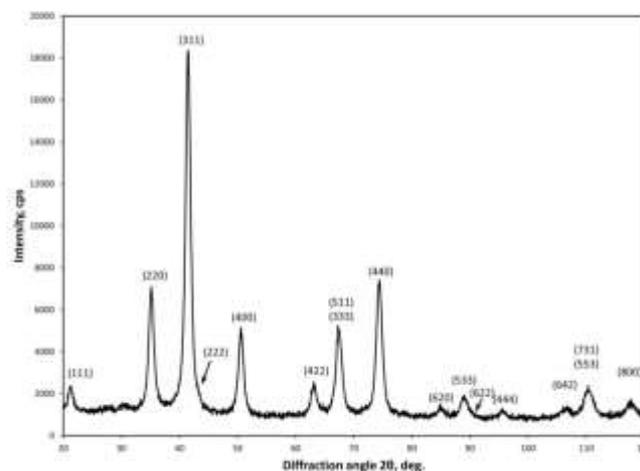


Fig. 2 Diffraction pattern of magnetite nanoparticles (9 ± 2 nm).

Hysteretic parameters at room temperature for magnetite nanoparticles of different sizes are listed in table 2. Ferromagnetic behavior was observed for all samples. In general, the saturation magnetization and coercivity values decrease as the particle size decreases. Thus, magnetization values vary between 60 and 80 emu/g, close to the theoretical values reported for bulk magnetite at room temperature [19] and the coercivity values

increases from 33 to 147 Oe, which is close to the reported value considering that the magnetization rotation takes place by a coherent mechanism following the Stoner-Wohlfarth model [19].

Table 2 Magnetic properties of magnetite nanoparticles

Nanoparticles	Size (TEM)	Magnetization, emu/g	Coercivity, Oe
Spheres	9 ± 2 nm	63	33
Spheres	22 ± 4 nm	55	35
Cubes	40 ± 5 nm	58	107
Spheres	50 ± 6 nm	80	147

Considering the application of these samples for further biomedical applications and the fact that colloid stability is the important property and most applications require the use of this material in the form of aqueous colloidal suspensions, it is worth studying the magnetite surface chemistry and the stability of the particles in water.

For the synthesis of the PEG-silane ligand, in the first step we carry out the reaction between 3-aminopropyltriethoxysilane and succinic anhydride in dry dioxane [16] to obtain compound **1** (fig. 3). In the second step, poly(ethylene glycol) 2-aminoethyl ether acetic acid (molecular weight 2500) reacts with compound **1** in dry methanol to give compound **2** (PEG-silane). To modify magnetic nanoparticles with PEG-silane we suspended magnetic nanoparticles in methanol solution of compound **2** with stirring during 24 hours. Then functionalized nanoparticles were collected by magnetic precipitation and excess ligand was removed by repetitive washing with methanol and water.

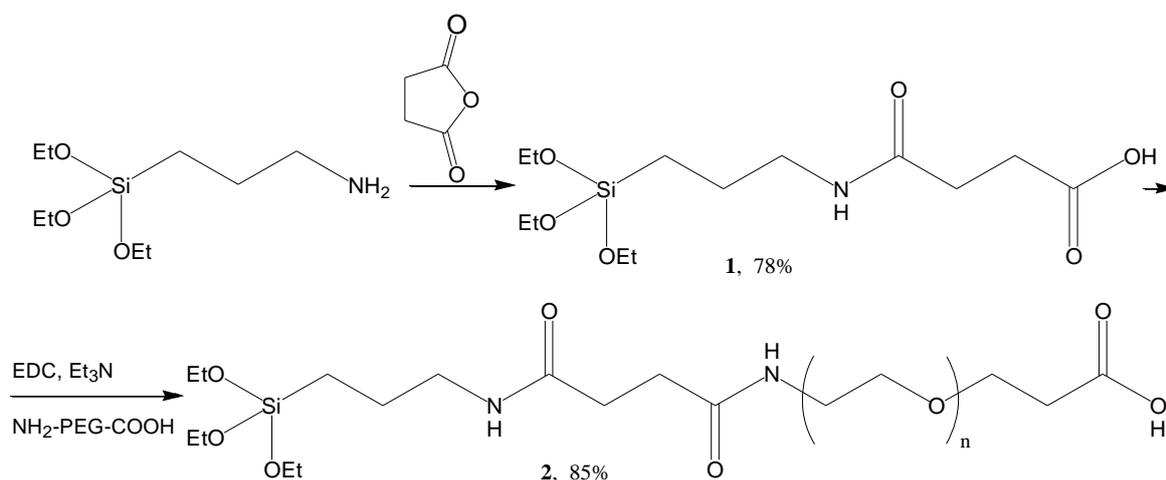


Fig. 3 Scheme of the synthesis of PEG-silane

Magnetite nanoparticles were transferred into a stable aqueous phase after PEG-silane functionalization, the hydrodynamic particle size of PEG-silane functionalized iron oxides (IO) for all samples became smaller (table 3) and stable for a long time (1 week). For example, big spheres without coating have a diameter of 250 nm by DLS, after coating the diameter decreased to 76 nm.

Table 3 Dependence DLS-diameter with and without PEG-silane coating iron nanoparticles (np)

nanoparticles	Diameter (TEM)	Diameter (DLS) (uncoated Np)	Diameter (DLS) (PEG-silane np)
Spheres	9 ± 2 nm	231 nm	36 nm
Spheres	22 ± 4 nm	475 nm	54 nm
Cubes	40 ± 5 nm	689 nm	92 nm
Spheres	50 ± 6 nm	251 nm	76 nm

To demonstrate the possibility using prepared magnetite nanoparticles as T2 agents we made MRI measurements. In an aqueous solution, MR images for the aqueous solutions of various nanoparticle concentrations were obtained. Samples of varying concentrations (ranging from 0.001 to 0.1 mM of iron) of nanoparticles in deionized water were prepared and put into microfuge tubes for imaging. The relaxivities of the PEG-silane iron oxide nanoparticles are presented in Table 4. If we compare the commercial T2 contrast agent with our materials synthesized PEG-silane magnetite nanoparticles are highly efficient T2 contrast agents.

Table 4 Relaxivity of PEG-silane magnetite nanoparticles

Sample	nanoparticles	Size	Relaxivity, T2, 1/mmol/s
5	Spheres	9 ± 2 nm	109
6	Spheres	22 ± 4 nm	140
7	Cubes	40 ± 5 nm	130
8	Spheres	50 ± 6 nm	189
Ex	Feridex ©	40 nm	126

CONCLUSION

Magnetite nanoparticles with different morphology were synthesized in water solution and were functionalized PEG-silane, which were synthesized previously. The magnetite nanoparticles were spherical and cubic in the TEM images, and showed uniform size distribution. Prepared water dispersible iron oxide nanoparticles may potentially use as a T2 contrast agent in MRI. The relaxivity T2 was higher to those of commercial contrast agents. The iron oxide nanoparticles presented here show great promise as nanomaterials for biomedical application due to inert coatings, easily conjugated to biomolecules and stability.

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