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Microwave-assisted synthesis of ultra-small iron oxide nanoparticles for biomedicine

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1. Details on Materials and Methods

Iron(II) chloride ($FeCl_2$, 98%), Iron(III) chloride ($FeCl_3$, 97%), Ethylene glycol (EG, $HO{-}CH_2{-}CH_2{-}OH$, 99%), Poly(ethylene glycol) (PEG, $HO{-}(CH_2{-}CH_2{-}O)_n{-}H$, BioUltra, 200), gold NPs (5 nm diameter, stabilized suspension in 0.1 mM PBS, reactant free, NPs concentration – $5.5{\cdot}10^{13}$ NPs/mL) were purchased from Sigma-Aldrich. Ultra-dry Samarium chloride ($SmCl_3$, 99.9%(REO)) and Sodium hydroxide (NaOH, 98%) were bought from Alfa Aesar. Reagents were used as such without further purification. Ultra-pure deionized water (18 MΩ·cm) was obtained at SimplicityUV system.

X-ray diffraction (XRD) pattern was recorded in 2θ interval from 25 to 65° with 0.02° step on ARL X'TRA powder diffractometer (Thermo Scientific, USA) using Cu Kα radiation (λ = 0.154056 nm). The average size of crystallites and lattice parameter was calculated by Rietveld analysis of the diffraction pattern with Jana2006 software. Independently, the average size of crystallites was also calculated by applying the Debye-Scherrer equation[1] to the (311) reflection on the diffraction pattern. X-ray Fluorescence (XRF) analysis was performed on M4 Tornado Micro-XRF spectrometer (Bruker, USA). A fraction of sample was gently pressed on surface of a boric acid disk. Transmission electron microscopy (TEM) images were acquired at Titan 80-300 microscope (FEI, Japan). A fraction of sample in isopropanol was dropwise casted on a copper mesh, covered with a lacey carbon. About 200 particles were counted on different TEM images to build a particle size distribution histogram. The Mössbauer spectra were measured on MS-1104Em rapid spectrometer equipped with Closed Cycle Refrigerator System CCS-850 (Janis, USA); the data were analyzed with UNIVEM software. The Fourier Transform Infrared (FTIR) spectrum was measured on FSM-2012 spectrometer (Infraspec, Russia) with resolution of 2 cm^{-1} in transmittance mode. The fraction of sample was mixed with KBr in 1:200 weight ratio and pelleted into 1 mm thick disk with a manual hydraulic press. Optical spectrum was recorded on UV-2600 spectrophotometer (Shimadzu, Japan). A fraction of sample in ethanol solution was measured against pure ethanol in 2 mm quartz cells. Magnetic properties were probed on a vibrating sample magnetometer 7400 (Lake Shore Cryotronics, USA) at room temperature. A fraction of sample was filled into a special plastic holder.

Cytotoxicity test towards HeLa cells was performed using the trypan blue exclusion assay in three different days, two biological replicates per day, two technical replicates per biological replicate for each nanoparticle sample. The cells were subcultured in T25 flasks, and further cultured for analysis in 96-well plates (SPL Lifesciences, South Korea) in the GlutaMax DMEM medium (Thermo Fisher Scientific, USA) supplemented with 10% of fetal bovine serum (GE Healthcare, UK) and 0.05 ug/ml of gentamicin (Biokhimik JSC, Russia). The cells were kept at 37 ℃ and 5% CO_2, with passive

humidification in the Sanyo MCO-18AC incubator (Panasonic, Japan). Cell viability data were analyzed for normality (the zero hypothesis was retained in all cases) using Kolmogorov-Smirnov test and for statistical differences using both non-parametric Mann-Whitney test and parametric ANOVA. The tests were cross-consistent.

2. Details on Experimental results

2.1. XRD analysis

The XRD pattern of the S1 sample contains the peaks at 2θ = 30.1°, 35.5°, 43.1°, 53.7°, 57.1° and 62.9°, which were indexed to (220), (311), (400), (422), (511) and (440) reflections. This sequence of reflections is typical for a spinel phase of the cubic crystal system, which is similar for iron oxides of magnetite and maghemite phase. The Rietveld refinement of diffraction pattern for S1 sample allowed to calculate the lattice parameter a = 8.369(2) Å. This parameter for pure γ-Fe_2O_3 is known to be a = 8.339(6) Å (JCPDS card 39–1346). The difference in values is caused by some expansion of crystalline lattice of S1 sample due to accommodation of Sm^{3+} ions with larger than Fe^{3+} ionic radius.[2]

2.2. Mössbauer spectroscopy

Table S1 Parameters of components (sextets) derived from Mössbauer spectrum of iron oxide sample measured at 13 K.

Sextet	δ, mm/s	ε, mm/s	B_{ef}, T	S, %	G, mm/s
S#1	0.54	0.02	51.7	50	0.78
S#2	0.35	-0.05	50.9	30	0.75
S#3	0.57	-0.07	46.0	20	1.04

Notes: δ – isomer shift, ε – quadrupole shift, B_{ef} – effective magnetic field on ^{57}Fe nucleus, S – sextet area, G – line width.

2.3. IR and UV-Vis spectroscopies

The FTIR spectrum‡ of the sample (mixed with KBr) is shown in Fig. S1a together with a reference spectrum of KBr. The comparison tells, that bands at about 3400 and 1600 cm^{-1} come from vibrations of water molecules physisorbed on KBr. The envelope at 1040 cm^{-1}, which is also shown in the inset in Fig. S1a with an example of fitting of its components contains C–O bond vibration with some participation from C–H bending vibrations. The bands of COO^- stretching modes can be identified at higher frequencies. Manifestations of methyl and methylene groups are visible around 2850-2900 cm^{-1}, partially hidden by a wide band from physisorbed water. The band at 592 cm^{-1} relates to Fe–O bond.[3] Such spectral sequence was already observed for PEG-covered iron oxide NPs with evidence of polyols partial oxidation.[4]

Optical spectrum‡ of iron oxide NPs is given in Fig. S1b, which is a typical decreasing curve. The positions of weak bands can be seen more clearly from a derivative (shown light gray in Fig. S1b). This sequence of band positions is typically observed for maghemite.[5] The only exception is the band at ~550 nm, which might be attributed to the surface plasmon absorption of residual gold NPs (their spectrum in stock solution is shown gray in Fig. S1b).

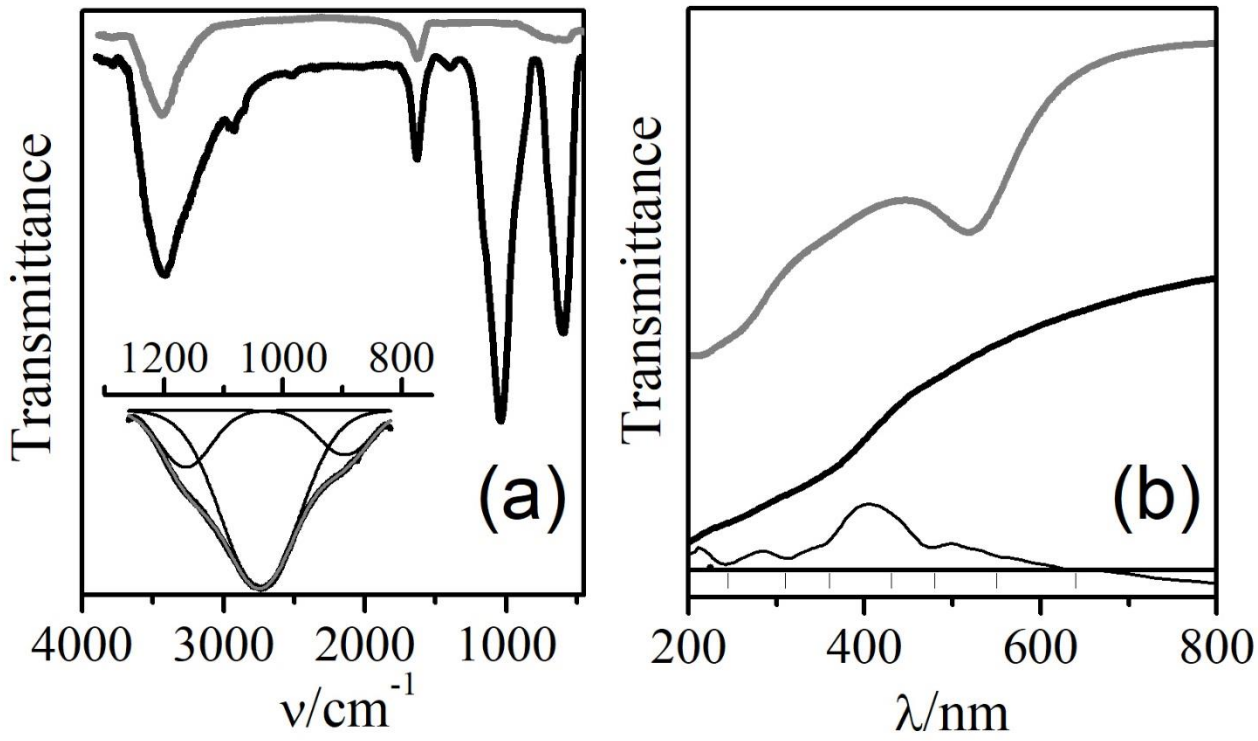

Figure S1 FTIR (a) and UV-Vis (b) spectra of iron oxide NPs (bold black). Additionally, there are the KBr spectrum (gray) and the fitting of a complex band at 1040 cm^{-1} (in the inset) in panel (a); there are derivative from the sample's spectrum (thin black) and spectrum of initial solution of Au NPs (gray).

2.4 Cytotoxicity test

Table S2 Resulting cell viability of the cell line HeLa after incubation with different concentration of iron oxide NPs in physiological saline compared to control solution.

Test solution	Number of samples	Cell viability (proportion ± error of proportion)	MW p-level; ANOVA p-level*
Control (NaCl 0.89%)	10	0.79 ± 0.04	--
25 μg/mL	7	0.79 ± 0.08	0.596; 0.775
50 μg/mL	6	0.76 ± 0.09	0.462; 0.379

*****_Note_: the NPs test solutions are only compared to the control group.

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