Hybrid composites based on alumina and magnetite nanoparticles for biomedical application

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Abstract. Porous nanostructured finds a great number of applications in science and industry. One of the most promising area of application of such materials lies in the field of drug delivery and creation of targetable nanoformulations. Here we describe a convenient and scalable method of production of magnetic porous matrices for drug delivery. The materials were created of stable boehmite and magnetite hydrosols via room-temperature sol-gel transition. As the result mesoporous hybrid matrices were produced with their textural and magnetic properties dependent on the ratio of components in their composition. The materials showed excellent biocompatibility on HeLa cell line in concentrations up to 200 µg/mL and good loading capacity with a model drug doxorubicin.

1. Introduction
Composite sol-gel nanomaterials with multifunctional properties are of great interest for application in various fields of science and industry. Due to high relative surface areas and porous structures such materials finds their applications as optical materials, heat and acoustic insulators, adsorbents, drug carriers and others, and the number of potential applications is constantly growing. Several key properties are important when it comes to practical applications: Firstly, textural properties of the composites. In general, composites with more developed microstructure shows greater sorptive capacities. Secondly, the composition of the materials and their stability. This point becomes even more important when it comes to biomedical application of such systems due to strict regulation of the materials applied in medicine. Thirdly, synthetic availability, scalability, and the cost of production of the nanostructured systems. Another beneficial property of nanocarriers is the responsivity to external factors, that may allow to navigate such systems or to influence their sorptive properties. For instance, this property can be used to separate adsorbents, concentrate drug carriers or to trigger a release or an adsorption process.

A number of nano-adsorbents have been described, based on metal oxides, hybrid materials, biomaterials, carbon, and others [1-6]. One of the special cases is magnetic nanosorbents based on magnetite, offering possibility to readily collect the used sorbent by an external magnetic field. While being rather convenient in application, magnetite-based sorbents mainly show relatively poor texture properties and hence low sorption capacities, which limit their potential applications.

In this work, we describe composite magnetic sorbents based on magnetite NPs (ferria) and boehmite NPs (alumina), showing both high magnetization values and excellent texture properties. Both of these materials have a long history of medical application and are known to be highly biocompatible. These two oxides can be mixed in any ratio to produce composites with variable properties most suitable for specific application scenarios.
2. Experimental details

2.1. Chemicals
Iron(II) chloride tetrahydrate \(\geq 98.5\%\), iron(III) chloride hexahydrate \(\geq 99\%\), aqueous solution of ammonia \(\geq 27.5\%\), aluminum isopropoxide \(\geq 99\%\), potassium dichromate, sodium hydroxide and doxorubicin were obtained from Sigma-Aldrich. HeLa cells, EMEM medium, Versene solution, MTT dye were obtained from Biolot.

2.2. Synthesis of alumina hydrosol
3.3 g of aluminum isopropoxide was added to deionized water pre-heated to 85 °C under constant stirring at 400 rpm and mixed for 15 minutes. Then, the resulting suspension was subjected to ultrasonic treatment (37 kHz, 110 W) under constant stirring at 300 rpm for 180 min. Mass concentration of the resulting alumina sol was 2 % [7].

2.3. Synthesis of ferria hydrosol
2.5 g FeCl\(_2\)·4H\(_2\)O and 5 g FeCl\(_3\)·6H\(_2\)O were dissolved in 100 mL of deionized water. Then, 11 mL of aqueous ammonia solution was added in a single portion this solution under constant stirring 500 rpm at room temperature and mixed for 5 minutes. The resulting black material was separated with a permanent magnetic and washed with deionized water until pH 7. The washed black precipitate was mixed with 100 mL of deionized water and subjected to ultrasonic treatment (37 kHz, 110 W) under constant stirring at 300 rpm for two hours. Mass concentration of the resulting ferria sol was 2 % [8].

2.4. Synthesis of ferria-alumina composites
For the preparation of the composite materials, (0.75-0.25) mL of the freshly prepared ferria hydrosol was added to the (0.25-0.75) mL of alumina hydrosol and vigorously mixed mechanically. Mixed hydrosol was evaporated at 60 °C at normal pressure and the resulting xerogel was ground in a mortar.

2.5. Cytotoxicity studies
HeLa cells were cultured in EMEM medium containing 5% FBS and gentamicin 50 g/mL at 37 °C in 5% CO\(_2\) in a humidified incubator (SANYO MCO 8 AC). Versene solution was used for cells detachment. Cells were seeded in density 5x10\(^3\) cells per well in 96-well plates overnight. Cells were incubated with aggregates dissolved in the medium supplement for 72 hours. Two-fold dilutions of MPS were used (final concentration 13-200 \(\mu\)g/mL). Cells were incubated with 150 \(\mu\)L of 0.5 mg/mL MTT in PBS at 37 °C in 5% CO\(_2\) for 1.5 hours. After MTT solution was aspirated and the blue-violet formazan crystals were dissolved by adding 200 \(\mu\)L DMSO and incubated on the shaker for 15 minutes. Each well was centrifuged for 5 min at 14 RPM for MPS precipitation. 100 \(\mu\)L of supernatant was transferred to the new 96-well plate. The optical density was measured at 570 nm wavelength using a microplate reader.

2.6. Characterization
Zeta potential was measured using a Photocor Compact-Z analyzer. The crystalline phase and crystallinity of the samples were measured by X-ray diffraction (Bruker D8 Advance) using Cu Ka radiation (\(\lambda = 1.54 \text{ Å}\)). The samples were scanned for 2h at a rate of 0.5 degrees per minute. The particle morphology and size were investigated by electron microscopy using a VEGA3 TESCAN scanning electron microscope (SEM) equipped by an X-Act EDX detector. Surface area, pore volume and pore size distribution were investigated using Quantachrome Nova 1200e by nitrogen adsorption at 77 K and analyzed by the BET and BJH equations. Prior to analysis, all samples were degassed at 110 °C for 4 hours. The samples for transmission electron microscopy (TEM) were obtained by dispersing a small probe in ethanol to form a homogeneous suspension. Then, a suspension drop was coated on a copper mesh covered with carbon for a TEM analysis (FEI TECNAI G2 F20, at an operating voltage of 200
kV). Spectrophotometric measurements were carried out using an Agilent Cary HP 8454 Diode Array spectrophotometer with TEC. Microplates were measured at Tecan Infinite F50 microplate reader.

3. Results

3.1. Synthesis and characterization of the composite materials

For the production of alumina-ferria composites, stable hydrosols were used, prepared by the US-assisted synthetic procedures described in details in our previous works [9-10]. The key feature of these systems is the absence of any surfactants in the nanoparticle composition and neutral pH level of the final sols combined with the excellent colloidal stability of the systems. The stability of the sols is achieved due to high zeta potential of the nanoparticles and dictated by the shift of the isoelectric point of the materials after ultrasonication. High intensity of US irradiation leads to surface hydroxylation and shifting isoelectric point of the materials to higher values (from pH = 8.3 to pH = 10 for alumina and from pH = 6.8 to pH = 8.0 for the ferria). Shift of isoelectric point and enrichment of the NP surface by hydroxyl groups leads to high values of the NP zeta-potential even at neutral pH levels, resulting in the formation of stable hydrosols (figure 1a, e).

![Figure 1](image_url)

Figure 1. Alumina (a-d) and ferria (e-h) hydrosols used in the study. Visual appearance of the materials of the as-synthesized alumina (a) and ferria (e); XRD spectra of the alumina, reference JCPDS file No. 21-1307 shown as blue lines (b), and ferria, JCPDS file No. 19-0629 shown as red lines (f); HR-TEM image of the alumina (c) and ferria (g) samples, interplane space is shown in the insert; HR-SEM image of alumina (d) and ferria (h).

Alumina hydrosol consisted of boehmite nanoparticles with an average crystallite size of 5×5×2 nm according to TEM (figure 1c) and SEM (1d). The system showed a narrow size distribution of particles with an average hydrodynamic diameter of 74 nm according to the dynamic light scattering technique. Having at neutral pH a zeta-potential value of +42 mV, boehmite nanoparticles formed a jelly-like hydrosol that remains stable without precipitation for a long period.

In its turn, ferria hydrosol consisted of magnetite nanoparticles with an average mean particle diameter of 10±2 nm and truncated octahedron morphology according to TEM (figure 1g) and SEM (figure 1h). DLS analysis demonstrated a narrow size distribution of the magnetite nanoparticles in the hydrosol with a mean hydrodynamic radius of 32 nm. Due to the high value of the zeta potential (+36 mV at pH = 7.4) magnetite nanoparticles form stable hydrosol showing magnetic fluid-like behavior, being attracted to an external magnetic field with dispersion phase. Due to the high colloidal stability and alike charge ferria and alumina hydrosols can be mixed in any ratio without mutual destabilization.
ensuring high degree of the colloidal solution homogeneity without signs of precipitation even after one week on a shelf (Figure 2).

Figure 2. Ferria and alumina hydrosols and their mixtures: visual appearance. Following a decrease in the ferria mass fraction, attraction of the hydrosol to the magnet is decreasing.

Due to the absence of surfactants and peptisation agents both ferria, alumina and their blends showed an ability to undergo sol-gel transition upon solvent removal and formed mesoporous xerogels at mild conditions. During that process removal of water molecules and partial dehydration of the nanoparticles surface occurred and interparticle hydrogen bonds are formed accompanied with van der Waals interaction, resulting in formation of rigid solid bulky materials. Investigation of the formed xerogel matrices by the TEM and SEM techniques showed uniform distribution of the components in the material (Figure 3a-c). Homogenous coloring of the energy-dispersive X-ray mapping proves the absence of aggregation in the process of mixing or solvent removal (Fig.3b). XRD spectra of the composite show peaks associated with both materials (compared to JCPDS files 04-002-3668 and 01-074-2898) (figure 3d-f) [11-12].

Figure 3. Characterization of ferria-alumina composite matrices. An HR-SEM image of the ferria-alumina 1:1 composite (a); an EDX image of the ferria-alumina 1:1 composites (b); a TEM image of the ferria-alumina 1:1 composite (c); XRD spectra of the ferria-alumina composite with mass fractions of ferria and alumina varying from 3:1 to 1:3, respectively.(d-f).

The textural properties of the formed xerogels depended on the initial ratio between the components and varied in the range between the values for individual ferria or alumina (Table 1).
Table 1. Textural properties of the composite materials

| System                  | Surface area, m²/g | Average pore diameter, nm | Total pore volume, cm³/g |
|-------------------------|--------------------|---------------------------|--------------------------|
| ferria                  | 121                | 8.6                       | 0.261                    |
| ferria – alumina 3:1    | 180                | 5.6                       | 0.264                    |
| ferria – alumina 1:1    | 265                | 4.4                       | 0.279                    |
| ferria – alumina 1:3    | 348                | 3.7                       | 0.296                    |
| alumina                 | 382                | 3.2                       | 0.301                    |

Due to the presence of magnetic nanoparticles the systems demonstrated superparamagnetic behavior with high saturation magnetization values (figure 4). Depending on the magnetite mass fraction the magnetization of the material reached up to 87 e.m.u./g at 8000 Oe for pure ferria xerogel and nearly linearly decreased with the reduction of magnetite mass fraction in the composite.

Figure 4. Magnetization curves of the composite materials.

3.2. Biocompatibility of the materials

Biocompatibility of the synthesized materials was investigated on HeLa cell line using the MTT assay. The composites were mechanically milled and filtrated through 0.22 µm syringe filter and incubated for 72 hours and after that cell viability was measured by staining with MTT (figure 5). It can be seen that all of the tested materials did not cause any significant cytotoxic effects on HeLa cells at a concentration range of 6.25-200 µg/mL. No significant differences between the indices of cell survival at different concentrations and no morphological signs of cell death were observed.

Figure 5. Cytotoxicity of the composites p < 0.05
3.3. Sorption kinetics
To investigate the potency of the composites as drug carriers a model drug doxorubicin was used to investigate the drug-loading capacities of the materials. For these purposes series of doxorubicin loading experiments were carried. The kinetic curves obtained for the doxorubicin adsorption from aqueous solutions onto composite nanoparticles are shown in figure 6a. Plotting \( t/q_e \) versus time (Figure 6b) yielded straight lines for the adsorption process demonstrating that sorption process mechanism can be described by the second-order equation:

\[
\frac{1}{q_t} = \frac{1}{k_2 q_e^2} + \frac{t}{q_e},
\]

where \( k_2 \) is the pseudo-second-order rate constant.

![Figure 6. Adsorption curves of doxorubicin on composite materials. Kinetic curve of adsorption (a); Pseudo-second-order kinetics for the adsorption of doxorubicin (b)](image)

In order to investigate the adsorption process, adsorption isotherms were modeled with the Langmuir equation (Table 2) [13]. The adsorption isotherm parameters and correlation regression coefficients are summarized in Table 1.

| System                  | \( q_m \) mg/g | \( K_L \) | \( R^2 \) |
|-------------------------|----------------|---------|---------|
| ferria                  | 23.51          | 22.41   | 0.951   |
| ferria – alumina 3:1    | 33.41          | 25.97   | 0.964   |
| ferria – alumina 1:1    | 43.65          | 27.31   | 0.941   |
| ferria – alumina 1:3    | 52.87          | 30.45   | 0.971   |
| alumina                 | 62.41          | 35.78   | 0.961   |

It can be seen that the Langmuir model fits with the experimental data with high \( R^2 \) values (> 0.941). Adsorption capacity of materials gradually increases with an increase in alumina mass fraction, in correlation with a change in the specific surface area determined by physical nitrogen adsorption. Compared to other adsorbents reported earlier, the composites show significantly better sorption capacities which can be explained by larger surface area and higher saturation of the surface by hydroxyl groups due to synthetic conditions.

4. Conclusions
Here we described the synthetic procedure for creation of alumina-ferria composites and physicochemical properties of the materials. Highly stable hydrosols of magnetite and boehmite can be synthesized by ultrasonically assisted coprecipitation method. Due to excellent colloidal stability and absence of surfactants these materials can be mixed in any ratio and condensed by sol-gel transition into
homogenous mesoporous materials. Textural properties and magnetization of the produced materials were dependent on the ratio between magnetite and boehmite and can be varied in the wide range. The materials demonstrated excellent biocompatibility and did not show cytotoxic effects in concentrations up to 200 µg/mL. The materials demonstrated good drug loading capacity up to 62 mg/g of doxorubicin and can be potentially used for magnetically-targeted drug delivery.

Acknowledgment
This work was supported by the Russian Foundation for Base Science grant 18-33-01170 mol_a

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