"Anisotropic magnetite nanoparticles: synthesis, study of physical and biological properties, and assessment of the prospects for use in MRI diagnostics"

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Abstract. Magnetic nanoparticles (MNPs) are increasingly used in various fields of biomedicine, for example, as a platform for targeted drug delivery, in hyperthermia for converting the energies of external electromagnetic fields into thermal energy, or in MRI diagnostics. Despite the fact that it has been repeatedly shown earlier that the shape and size of nanocrystals of MNPs have a significant effect on their magnetic and relaxation properties, there are still no fundamental experimental studies that would establish the relationship between the type of MNPs, their physical characteristics, in vitro toxicity, biodistribution in the body and contrasting properties in MRI diagnostics of various experimental tumor models. T2-contrast agents (CA) for MRI based on MNPs of complex iron oxides are a safe alternative to T1-CA, which are presented by chelate complexes Gd^{3+} and Mn^{2+}. In turn, the use of magnetite (Fe_3O_4) MNPs of various morphologies as CA is primarily due to biocompatibility, low toxicity, and pronounced magnetic properties of such a material. Most of the experimental works describing the prospects for the use of Fe_3O_4 MNPs in MRI were carried out using the example of spherical MNPs, while the most attractive CA are MNPs with a pronounced shape anisotropy, which can be changed to control the magnetic properties of the material. The development of new methods for the synthesis of anisotropic MNPs with controlled shape and size, including the subsequent assessment of the prospects for using such MNPs as an MRI-CA, will provide fundamental knowledge about the relationship between the type of MNPs - physical properties of MNPs - biological response, which determines the relevance of this work.

The main goal of the present work was to develop methods for the synthesis of anisotropic MNPs of complex iron oxides with controlled morphology, as well as to study the properties of such MNPs in order to obtain fundamental knowledge about the relationship between the type of MNPs - physical properties of MNPs - in vitro and in vivo properties of MNPs - contrasting effect in MRI - experiments.

The main tasks were:

1. Development of methods for the synthesis of anisotropic MNPs of complex iron oxides with controlled shape and size;

2. Investigation of the structure and morphology of MNPs by TEM, XRD analysis, IR-spectroscopy, including investigation of their static and dynamic magnetic properties;

3. Development of a method for obtaining stable aqueous colloids of MNP under physiological conditions;

4. Investigation of the cytotoxicity of MNP of various shapes and sizes *in vitro*, study their biodistribution in animals with experimental tumor models, and assessment of the prospects for using such MNP as MRI-CAs.

Scientific novelty. For the first time, a method was developed for the synthesis of cluster magnetite nanoparticles (CLMNP) using aliphatic and aromatic cyclic carboxylic acids, and the effect of the latter on the shape and size of the resulting NPs was studied. It was shown that both the size of CLMNP and the size of individual crystallites of which they are composed decrease according to the logarithmic law with an increase in the lipophilicity constant in the series of cyclic aliphatic carboxylic acids. Measurements of the magnetic properties of CLMNP have demonstrated the phenomenon of the magnetostatic effect in the obtained cluster structures. In addition, an original two-stage technique for the synthesis of rod-shaped magnetite nanoparticles RSMNP using microwave radiation was developed, which allows the controlled reduction of a nonmagnetic precursor — rod-like acagenite NPs, to magnetic NPs without losing their shape. In the case of cubic magnetite nanoparticles (CMNP), it was shown that the size of their magnetic core can be controlled by adjusting the molar ratio of the iron-containing precursor to the surfactant, such as oleic acid and sodium oleate. It was shown that the size of the CMNP core decreases exponentially with an increase in the total amount of such surfactants in the reaction mixture. A detailed study of the influence of NPs morphology on their static and dynamic magnetic properties was carried out, and the corresponding functional dependences were obtained. In MRI experiments, it was shown that CMNP allow obtaining the highest values of the r2-relaxation rate of water protons in comparison with NPs of a different shape, which is several times higher than those for commercial CAs. In addition, for the first time, a comprehensive assessment of the influence of NPs morphology on their biodistribution *in vivo* was carried out.

The theoretical and practical significance of the work. The experimental techniques for the chemical synthesis of biocompatible anisotropic NPs developed in this dissertation can represent practical recommendations for the creation of highly efficient CAs to increase the efficiency of visualization of internal tissues and organs during MRI diagnostics. The effect of a pronounced CLMNP accumulation in the kidneys during their intravenous administration, discovered in this work, dictates the need for a more thorough study of the mechanisms of CAs pharmacodynamics based on MNPs of complex iron oxides, depending on their morphology and surface chemistry. In addition, data on the biodistribution of synthesized anisotropic NPs can serve as a basis for creating new types of spacecraft that are selective with respect to certain

targets. On the basis of the studies carried out, methods for producing spacecraft based on CLMNP and RSMNP were developed and patented (RF Patents No. RU2664062C2, 2018; No. RU2686931C1, 2019).

Defense Provisions.

1. The developed techniques for the synthesis of anisotropic NPs make it possible to obtain monodisperse cubic, cluster, and rod-like NPs in the size range 10 - 40 nm. The shape of NPs can be controlled by selecting a specific surfactant, and the size and monodispersity of the magnetic core can be controlled by the surfactant concentration, the duration of synthesis, and the heating rate of the reaction mixture.

2. Static and dynamic magnetic properties of NPs directly depend on their morphology and reach an optimum for CMNP with a magnetic core size of 15 - 20 nm;

3. The shape of NP is a key parameter that determines their biodistribution in the body during intravenous administration of the drug;

4. The combination of individual NPs into cluster structures leads to the appearance of new properties in the material due to the phenomenon of the magnetostatic effect.