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Effect of Magnetite Nanoparticles on Human Blood Components

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The qualitative composition and zeta potential of magnetite nanoparticles (size 4.2±1.2 nm) obtained by co-precipitation method were determined by X-ray and diffraction dynamic light scattering. The zeta potential of Fe_3O_4 particles was -15.1±4.5 mV. The possibility of interaction of magnetite nanoparticles with human blood plasma proteins and hemoglobin as well as with erythrocyte membranes was demonstrated by spectrophotometry, electrophoresis, and fluorescence methods. No changes in the sizes of hemoglobin molecules and plasma proteins after their modification by Fe_3O_4 particles were detected. The possibility of modifying the structural state of erythrocyte membranes in the presence of magnetite nanoparticles was demonstrated by means of fluorescent probe 1-anilinonaphthalene-8-sulfonate.

Key Words: *magnetite; hemoglobin; erythrocytes; blood plasma proteins; membranes*

Magnetic nanoparticles (MNPs) and nanosystems based on MNPs represent a promising material for application in medicine and nanobiotechnology [1]. Nanoscale size, magnetic properties, and biocompatibility [2-4] allow effective application of MNPs for targeted drug delivery, improved visualization in MRI, magnetic hyperthermia, detection of genetic mutations, and immunoanalysis [5-7].

The biological effects of nanoparticles largely depend on their size, shape, composition, charge, microenvironment, and other characteristics [8]. The ability of nanoparticles to aggregate (aggregation stability) is extremely important for assessing their state and activity in biological media (blood, tissue fluid, and lymph) [9,10]. To prevent aggregation and flocculation of magnetite, its surface is modified with various stabilizing agents [11]. Such coating also makes it possible to extend the time of particle circulation in the bloodstream. Adsorption of proteins on the surface of unstabilized MNPs affects penetration nanoparticles into cells and the mechanisms of their modifying effect on the subcellular components [12]. It is known that the effect of magnetite on human blood erythrocytes significantly depends on the MNP size. In particular, particles <10 nm in size have enhanced ability to induce ROS generation and to increase the rate of macrophage proliferation [13]. However, the detailed mechanisms of the action of MNP on human blood components are poorly investigated.

Here we studied the qualitative composition and zeta potential of synthesized magnetite particles, and assessed the possibility of their interaction with human hemoglobin, plasma components, and erythrocyte membranes.

MATERIALS AND METHODS

Obtaining magnetite nanoparticles. Magnetite nanoparticles were obtained by co-precipitation method

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using a mixture of iron (II) and (III) salts with a total concentration of 30 mmol/liter (stoichiometric ratio 1:2); 1% ammonia solution was used as a precipitating agent. The obtained particles were washed 2 times with distilled water by placing them into magnetic field (using a magnetic rack). Experimental samples of magnetite were collected in a Petri dish and dried in a FreeZoneTriad lyophilic dryer (Labconco).

The zeta potential of MNPs was measured by dynamic light scattering on a Zetasizer Nano ZSP particle characterization system (Malvern Instruments).

Analysis of the interaction of magnetite with donor blood plasma and hemoglobin. Hemoglobin was isolated from blood erythrocytes of donors (11 people) by the Drabkin's method. The study was approved by the Ethical Committee of Voronezh State University (Protocol No. 42-02a of April 10, 2023) and was conducted in compliance with ethical standards (WMA Declaration of Helsinki; paragraph 32) [14]. Hemoglobin was diluted in 0.1 M sodium phosphate buffer (pH 7.4) to a concentration of 5.5×10^{-7} M, the plasma was diluted to an optical density of OD_{275}=0.8. Hemoglobin and plasma were incubated with MNPs for 1 h (1 mg MNPs per 1 ml of hemoglobin or plasma solution). Then MNPs were precipitated by centrifugation (8000 rpm, 20 min) on a MiniSpin centrifuge (Eppendorf). The electronic absorption spectra of the supernatant were recorded on a Shimadzu UV-2401 PC automatic spectrophotometer in the wavelength range of 230-700 nm. Optical density was recorded using 1 nm step and 1 nm spectral slit width. Protein size was estimated on a Zetasizer Nano ZSP system.

Evaluation of hemoglobin adsorption on nanoparticles. Electrophoresis in SDS-PAAG was performed by the modified Davis method [15] using a Mini-Protean electrophoretic chamber (Bio-Rad). A buffer containing 0.125 M Tris-HCl (pH 6.8), 4% SDS, 20% glycerol, 0.002% bromophenol blue, and 10% β-mercaptoethanol was added to the protein samples before loading to the gel. The gel was stained with 0.01% Coomassie R-250 solution in an ethanol—acetic acid—water mixture (4:1:5). The image was processed using ImageJ software.

Analysis of the structural state of human blood erythrocyte membranes by fluorescent probe method. The blood diluted 1:1 with Hanks' solution (pH 7.4) was centrifuged at 3000 rpm for 10 min on a MPV-360 centrifuge (Med Instruments). The plasma and the top layer of leukocytes were collected. Erythrocytes were washed 3 times with saline. The erythrocyte suspension (2×10^6/ml) was incubated with MNP (1 mg/ml) for 1 h, after which the cells were precipitated. Erythrocyte membranes were obtained by hypoosmotic hemolysis. The hemolysate was centrifuged on a MiniSpin centrifuge at 13,000 rpm for 15 min. The membrane potential was assessed by fluorescence intensity of the negatively charged surface probe 1-anilinonaphthalene-8-sulfonate (ANS; Sigma). The intensity of ANS fluorescence in erythrocyte membranes was measured λ_{ex}=360 nm and λ_{em}=420-600 nm. The final ANS concentration in erythrocyte membrane suspension was 2.5×10^{-5} M. The probe fluorescence spectra were recorded on a Shimadzu RF-1501 spectrofluorometer.

Statistical analysis. The results were processed using Microsoft Excel 2010 and presented as *M*±*SD*. The significance of differences between the reference and experimental values was assessed using the Student's *t* test at $p<0.05$.

RESULTS

The synthesized MNPs had zeta potential -15.1±4.5 mV, which is consistent with the previously obtained data [16] on the negative charge of the magnetite particles surface at pH⩾4.5. The size of MNP, as we have previously established by transmission electron microscopy, was 4.2±1.2 nm [17].

To study the possible modifying effect of MNP on human blood components, changes in the structural state of hemoglobin molecules, plasma components, and erythrocyte membranes after exposure to magnetite particles should be evaluated. Absorption spectra of human oxyhemoglobin solutions in the presence and absence of MNP are presented in Figure 1, *a*. Solutions of intact hemoglobin samples had absorption maxima at wavelengths of 272, 348, 414, 541, and 576 nm. When analyzing the absorption spectra of human blood plasma before and after MNP exposure, no shift of the absorption maximum (278 nm) was also detected (Fig. 1, *b*). The decrease of optical density in the absorption maxima of hemoglobin and plasma can be related to the interaction of negatively charged magnetite surface and positively charged groups of proteins, as a result of which some proteins are sorbed on magnetite and precipitated during centrifugation.

To confirm the possibility of interaction between hemoglobin molecules and MNPs, electrophoregrams of intact MNPs-treated hemoglobin samples were obtained (Fig. 2). When constructing densitograms of the obtained images and determining the area under the curves characterizing the amount of protein, it was found that the protein level after magnetite exposure decreases by 9.9% as compared to that in the intact sample.

The decrease of hemoglobin level in the studied samples after their modification with magnetite particles is caused by the processes of interaction between protein molecules and nanoparticles leading to the formation of their complexes. No statistically

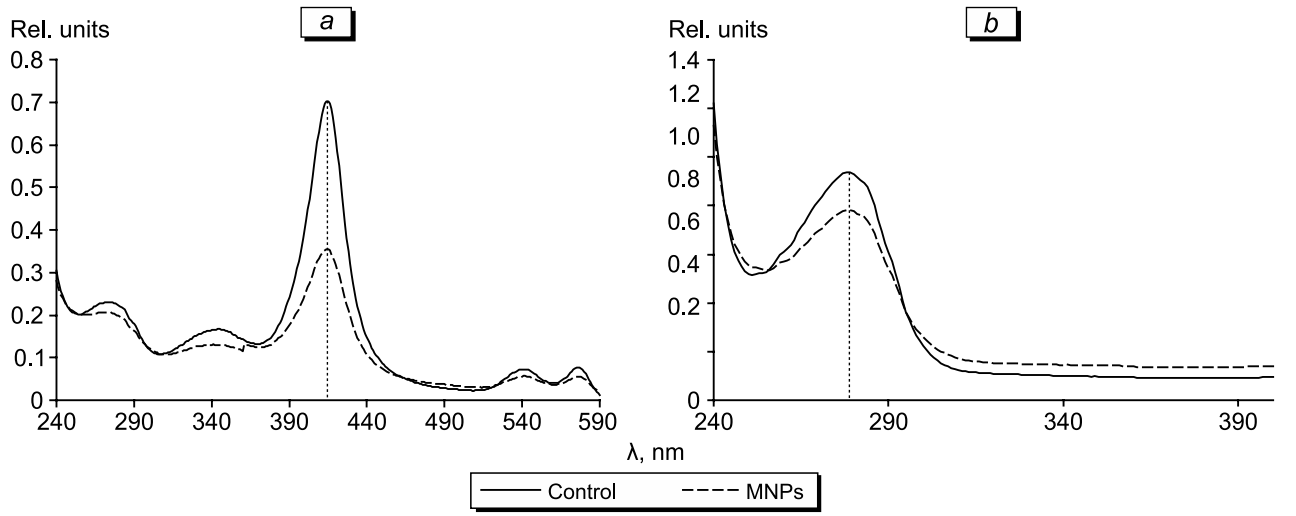

Fig. 1. Absorption spectra of hemoglobin (*a*) and human blood plasma (*b*) after incubation with magnetite nanoparticles.

significant change in the hydrodynamic diameter of proteins after 1-h incubation with MNPs was found in hemoglobin (8.1±1.3 nm) and plasma (6.7±1.4 nm) as compared to the control (6.1±0.9 and 8.0±1.0 nm, respectively).

The possible modifying effect of MNP on erythrocyte membranes was studied using the fluorescence probe ANS. The probe fluorescence spectrum in the presence of intact membranes (control sample) had a maximum at 494 nm, and after magnetite exposure at 509 nm (Fig. 3). The maximum fluorescence intensity for the control sample and modified erythrocyte membranes was 5.5±2.2 and 5.9±2.0 rel. units, respectively. The shift of ANS fluorescence maximum in the presence of erythrocyte membranes modified by MNPs to a longer wavelength region in comparison with native membranes suggests that the microenvironment of chromophoric groups becomes more polar. At the same time, the fluorescence intensity for the control and experimental samples practically did not differ. Apparently, there are no significant changes in the charge state of membrane structures after exposure of erythrocytes to MNP.

The fluorescence spectrum of ANS in the presence of intact membranes is asymmetric, which probably attested to interaction of ANS with both lipid and protein components of the membranes. The luminescence spectrum of the probe in complex with

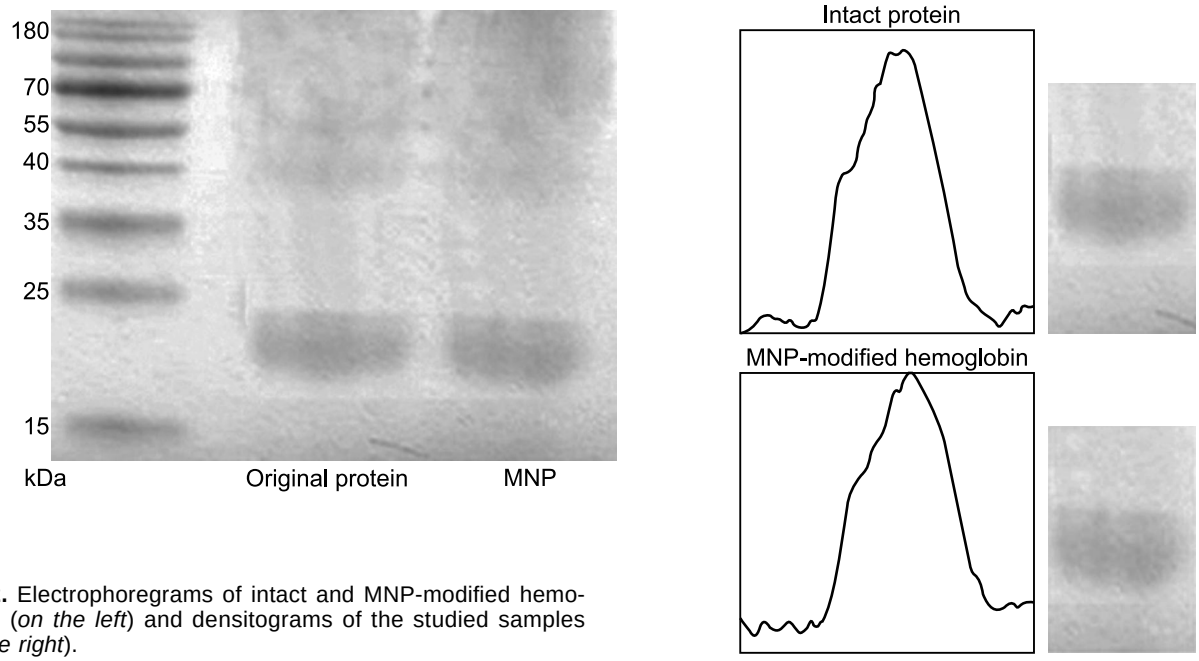

Fig. 2. Electrophoregrams of intact and MNP-modified hemoglobin (*on the left*) and densitograms of the studied samples (*on the right*).

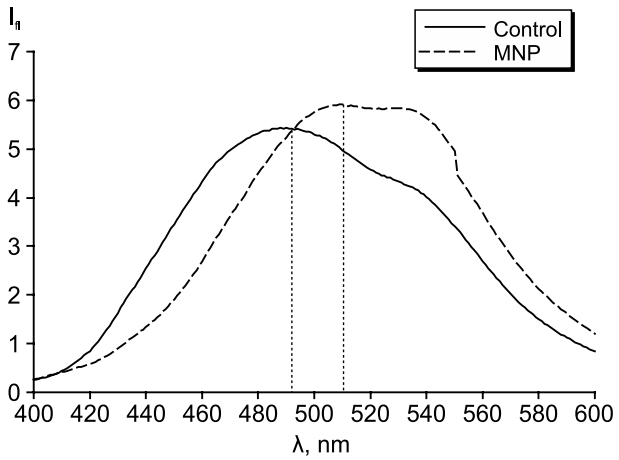

Fig. 3. Fluorescence spectra of ANS probe in the presence of intact and MNP-modified human erythrocyte membranes.

modified membranes differs from that in the reference samples: its shape and position of "branches" change. This may be due to the appearance of additional sites (groups) on the membrane surface after exposure to MNPs affecting the probe chromophore microenvironment and the shape of its fluorescence spectrum.

It is assumed [18] that erythrocyte membrane disturbances may occur due to mechanical interaction of magnetite with the membrane and the formation of ROS. It was previously shown [19] that direct contact between magnetite and phospholipids led to peroxidation of membrane lipids. The ability of MNP to cause damage to erythrocytes, leading to their apoptotic death, as well as its effect on rheological processes in blood was also demonstrated.

Thus, the synthesized magnetite particles are formed by Fe_3O_4 and have zeta potential of -15.1± 4.5 mV. They can interact with hemoglobin molecules and components of the plasma and erythrocyte membranes. We revealed no changes in the size of hemoglobin and plasma molecules after their modification by MNPs. However, magnetite nanoparticles can modify the structural state of erythrocyte membranes leading to disfunction of their components.

The obtained data on the MNP composition, properties, and interaction with human blood components suggest that they can be considered in general as a hemocompatible agent. However, biomedical use of unmodified magnetite nanoparticles should take into account their ability to form complexes with plasma proteins and hemoglobin, to change the state of erythrocyte plasma membranes. Such circumstances necessitate further studies of their potential practical application in nanobiotechnology and medicine.

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Conflict of interest. The authors have no conflicts of interest to declare.

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