Iron Oxide Nanoparticles for Isolating DNA from Blood Cells

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Abstract—Magnetic iron oxide nanoparticles for separating DNA from blood cells are synthesized. Magnetic nanoparticles with a silicate coating are obtained, and their physical and chemical properties are studied. The possibility of using the nanocomposites to isolate DNA from leukocytes for hematological studies is confirmed experimentally.

INTRODUCTION

Magnetic nanoparticles are of great interest in biology and medicine, both for fundamental research and for use in different fields [1–6]. Magnetic nanoparticles are now widely used to separate biological molecules due to the possibility of automating the process, its simplicity and relative safety, and the ability to obtain a pure product. Despite its advantages, however, this technology creates a number of difficulties. These include the high reactivity of metal oxides, the effect they have on molecules of nucleic acid, and the possibility of damaging the latter [7]. The biocompatibility of a magnetic nanoconstruction is thus an important characteristic that determines the applicability of the developed particles in biomedicine [8]. The effect the particles have on a target molecule depends on their chemical composition, size, concentration, and other parameters [9].

Iron oxide nanoparticles are among the compounds least aggressive to biological objects [10]. The most commonly used forms of iron oxide in biomedicine are magnetite (Fe_3O_4) and maghemite (γ-Fe_2O_3). According to the results in [11], particles with an average size of more than 60 nm exhibit the physical properties and crystal structure of massive magnetite with lattice parameter $a = 0.8383$ nm. Iron oxide nanoparticles of fairly small size are a nonstoichiometric compound of the magnetite–maghemite series. This compound can be described by the crystal chemical formula $Fe^{3+}[Fe^{2+}_{1-3n}Fe^{3+}_{1+2n}\phi_n]O_4$, where ϕ denotes vacancies, and n is their formula coefficient [11]. At sizes of less than 10 nm, the particles are similar to maghemite in composition and structure.

Modifying the surfaces of such particles with substances that have higher sorption capacity and chemical resistance allows us to obtain structures with high functional activity and minimal side effects on target molecules [12]. The simplest coating for the isolation of nucleic acids is silicate (SiO_2). Isolating DNA is one of the first and most important stages of clinical laboratory diagnostics in molecular genetic studies and a number of tests for the presence of infectious diseases. The material most commonly used is blood, so developing and optimizing ways of isolating DNA from blood cells remain relevant.

The aim of this work was to develop nanomagnetic structures based on iron oxide nanoparticles for separating DNA molecules from blood cells.

EXPERIMENTAL

Magnetic particles were obtained via coprecipitation from solutions of $FeCl_3$ and $FeCl_2$ salts in 2.7 : 1 and 2 : 1 molar ratios at room temperature. Weighed portions of the chemicals were dissolved in distilled water and added to an NH_4OH solution (25%) with stirring until the pH reached 10. Stirring continued for 4 h (for 2.7 : 1) and 30 min (for 2 : 1). Once the reaction was complete, the magnetic nanoparticles were in both cases collected using a magnet and washed sev-
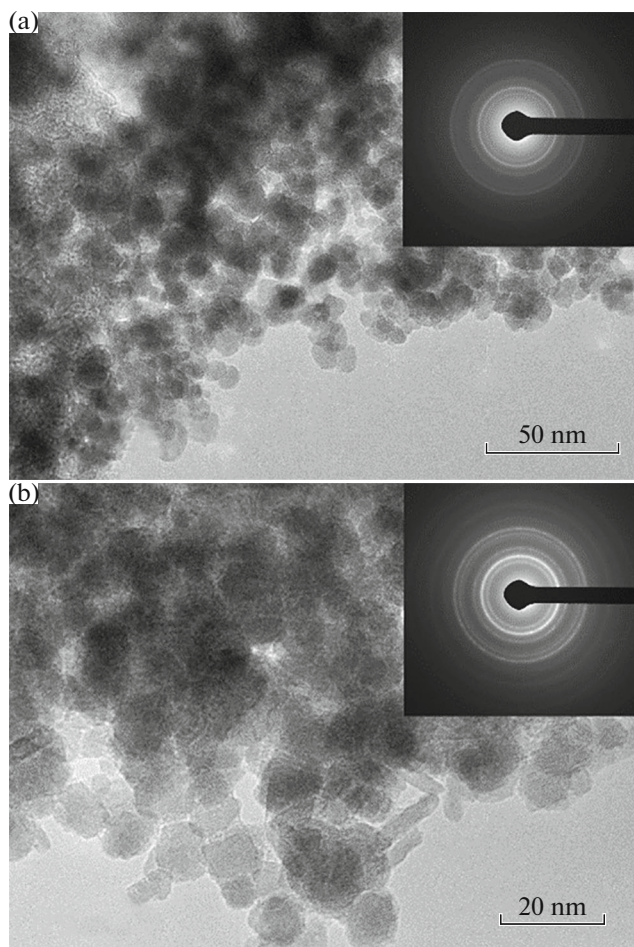
Fig. 1. Images and diffraction patterns of nanoparticles obtained with a high-resolution transmission electron microscope. (a) Iron oxide particles synthesized from a solution with a salt ratio of 2 : 1; (b) iron oxide particles synthesized from a solution with a salt ratio of 2.7 : 1.

Fig. 2. Mössbauer spectra. (a) Iron oxide particles synthesized from a solution with a salt ratio of 2 : 1; (b) iron oxide particles synthesized from a solution with a salt ratio of 2.7 : 1.

eral times in distilled water until the pH reached 7.0. The surfaces of the particles were modified with silica using tetraethoxysilane (TEOS) and 3-aminopropyl-triethoxysilane (APTES). The resulting nanoparticles were studied via transmission electron microscopy, IR spectroscopy, Mössbauer spectroscopy, and magnetometry.

RESULTS AND DISCUSSION

The electron microscope images in Fig. 1 show that nanoparticles synthesized from a solution with a salt ratio of 2 : 1 were spherical ($d \sim 10$ nm), while those synthesized from a solution with a salt ratio of 2.7 : 1 were in the shape of plates 11 nm in diameter and 2.5 nm thick. The insets in Fig. 1 show diffraction patterns in which all reflections characteristic of magnetite−maghemite nanoparticles were recorded.

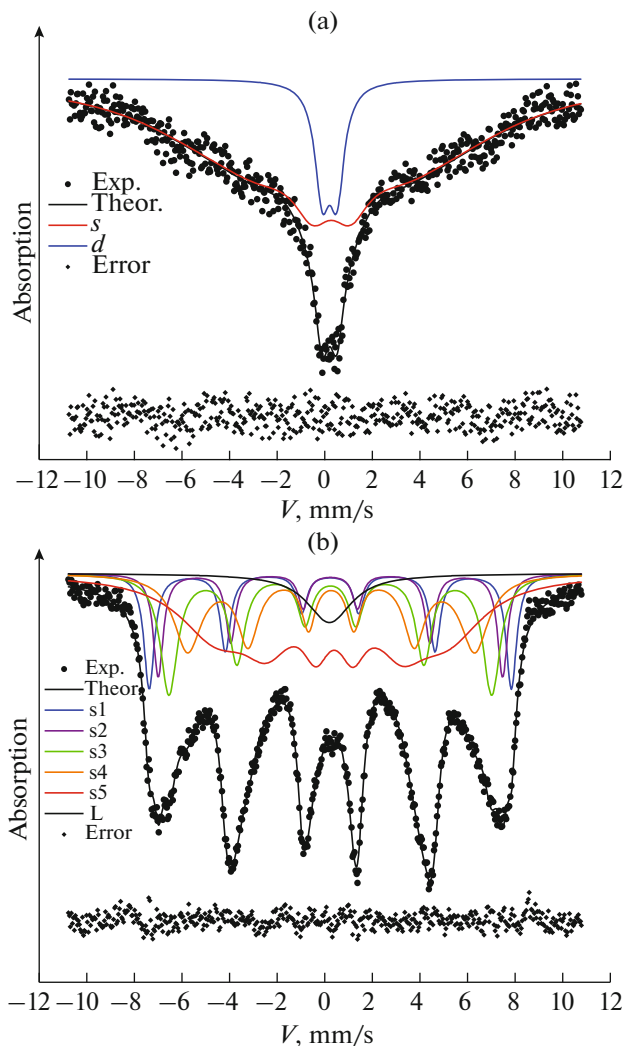Figure 2b shows the Mössbauer spectroscopy spectrum of nanoparticles synthesized from a solution with

a salt ratio of 2.7 : 1. Spectral components are represented by colored lines. The states of atoms in the superparamagnetic state are characterized in this spectrum by singlets. The states of atoms in the locked state are characterized by sextets. Table 1 shows the parameters of the detected nonequivalent positions of Fe^{3+} cations and their association. We estimated the thickness of the surface layer (5.6 Å) from the number of positions and the ratio $(A_5 + A_4 + A_3) = V_s/V$, where V is the total volume of a nanoparticle; V_s is the volume of its surface region; and A_3, A_4, and A_5 are the numbers of surface and near-surface positions according to Table 1 [13].

The model spectrum of nanoparticles synthesized from a solution with a salt ratio of 2 : 1 (spectrum a) was approximated by the sum of one sextet and one doublet. The result from fitting is summarized in Table 1. The values of the chemical shifts indicate that all of the

Table 1. Mössbauer parameters

	IS	H	QS	W_{34-16}	A	Position
(a)	0.34	472	0.01	0.53–0.53	0.11	Fe^{3+}, tetrahedral environment
	0.35	449	0	0.50–0.50	0.09	Fe^{3+}, octahedral environment
	0.34	421	0	0.71–0.87	0.19	Fe^{3+}, tetrahedral subsurface
	0.38	374	0	0.70–1.40	0.19	Fe^{3+}, octahedral subsurface
	0.45	303	−0.23	1.40–3.74	0.37	Fe^{3+}, surface
	0.31	–	–	2.26	0.05	Fe^{3+}, superparamagnetic particles
(b)	0.39	311	0	2.0–8.3	0.90	Fe^{3+}, ferromagnetic particles
	0.31	–	0.61	0.75	0.10	Fe^{3+}, superparamagnetic particles

IS the isomeric chemical shift. QS is quadrupole splitting, ± 0.02 mm/s. W is the absorption line width at half maximum, ± 0.02 mm/s. A is the fractional occupancy of the site, ± 0.03. H is the hyperfine field, kOe.

iron in the samples was in the trivalent high-spin state. Compared to sample (b), the sextets display very broad absorption lines with considerable broadening of the outer lines. The fluctuations of hyperfine fields over the sample positions range from 180 to 440 kOe.

Most of the iron (~90%) was in a magnetically ordered state at room temperature (sextet). A paramagnetic doublet accounts for ~10% of the total spectrum. The drop in the hyperfine field on the iron atoms of the magnetic phase relative to sample (b) could indicate a reduction in the efficiency or number of interatomic interactions (lattice disordering), or in the size of nanocrystallites. Along with the magnitude of the hyperfine field in this sample, the broadening of the resonance lines was due to relaxation effects (i.e., these particles were approaching the transition from superparamagnetic to ferromagnetic order at room temperature). The temperature of the transition to the superparamagnetic state (blocking temperature T_B ~ KV/k_B, where k_B is the Boltzmann constant) was determined by particle volume V and magnetic anisotropy K. The linear sizes of the two types of particles were relatively equal (see Fig. 1), but because particles (b) were in the form of plates, the contribution from shape anisotropy was added and the blocking temperature rose. The blocked particles were prone to the formation of agglomerates due to dipole−dipole interaction, and the specific surface area shrank. Superparamagnetic particles are more difficult to collect with a magnetic field, so particles in a ferromagnetic state with a T_B near room temperature are apparently the ones best suited for magnetic separation.

Figure 3 shows the FTIR spectra of magnetic nanoparticles: (a) is the spectrum of iron oxide particles synthesized from a solution with a salt ratio of 2 : 1, while (b) is that of iron oxide particles synthesized from a solution with a salt ratio of 2.7 : 1. Analysis of the IR spectra revealed the presence of Fe−O bonds (a, 580;
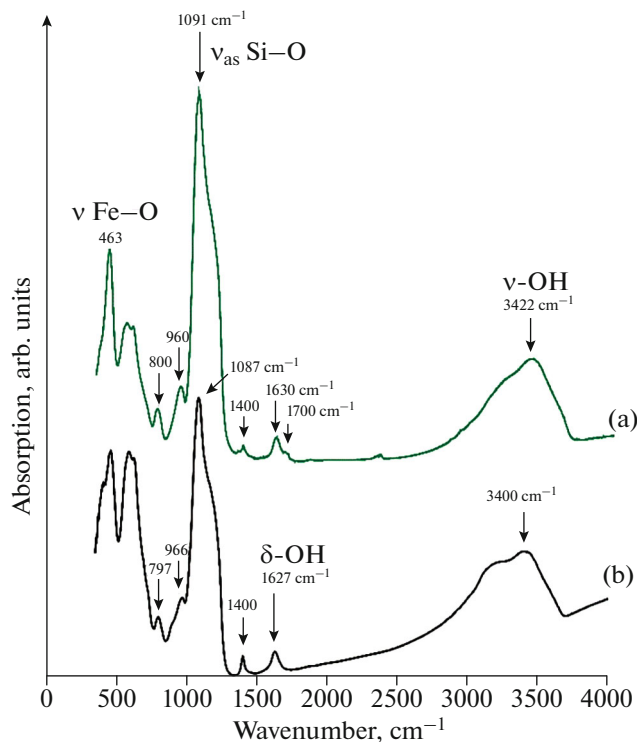

Fig. 3. Fourier-transform IR spectra. (a) Iron oxide particles synthesized from a solution with a salt ratio of 2 : 1; (b) iron oxide particles synthesized from a solution with a salt ratio of 2.7 : 1.
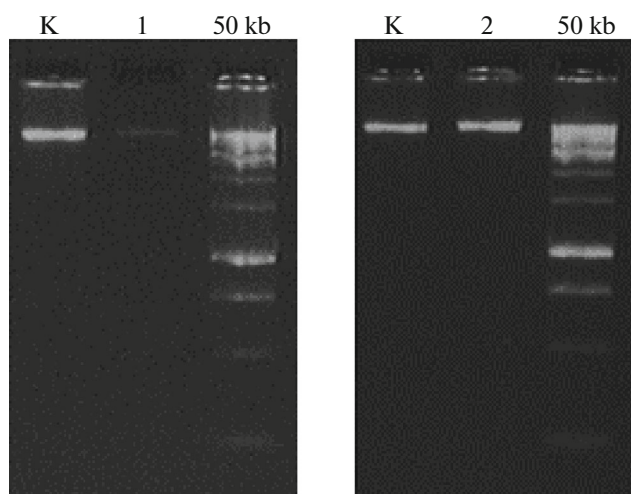
Fig. 4. Result from analyzing DNA isolated using MNPs : DNA electrophoresis in 1% agarose gel. K is the isolation control (silicate sorbent). (1) Particles synthesized from a solution with a salt ratio of 2.7 : 1; (2) particles synthesized from a solution with a salt ratio of 2 : 1. DNA molecular weight marker, 50 kb.

b, 590 cm^{-1}) in all samples. The peaks of strong stretching vibrations of siloxane groups Si−O (a, 1091; b, 1087 cm^{-1}) and silanol groups Si−OH (~800 and ~960 cm^{-1}) in the spectra of nanoparticles testify to the formation of silicon oxide shells on the surfaces of nanoparticle. The ν-OH stretching vibrations formed an intense band in the region of 3200−3600 cm^{-1} in the considered samples.

The Si−O peak in the spectra shifted from 1091 to 1087 cm^{-1} and became less intense. Deformation vibrations (~463 cm^{-1}) of the Si−O bonds also changed and were most intense in spectrum (a) (see Fig. 3). Such differences between spectra (a) and (b) could be due to the latter sample containing a high concentration of OH groups. It was established that siloxane groups participated in the bonding between magnetic particles and the silica layer, while silanol groups were most active on its surface and chemically active in the formation of covalent bonds. The presence of OH groups on the surfaces of silica particles can inhibit the hybridization of DNA molecules.

DNA was isolated from blood cells using the synthesized magnetic particles and solutions from a commercial kit of DNA-sorb-B reagents (AmpliSens, Russia). The complete set of DNA-sorb-B that included a silicate sorbent for DNA was used as the reference for isolation. The amount and quality of DNA obtained by using the magnetic nanoparticles synthesized from a solution of iron salts in a 2 : 1 ratio was comparable to those of DNA isolated using the silicate sorbent. Electrophoresis of the obtained DNA in agarose gel (Fig. 4) showed a single clear band of purified molecules, testifying to their integrity and lack of fragmentation and making the samples suitable for subsequent genetic studies. The intensity of the luminescence of the DNA's bands was proportional to its amount, showing that magnetic nanoparticles can isolate molecules of nucleic acid from a mixture in amounts comparable to using a standard silicate sorbent.

CONCLUSIONS

Iron oxide nanoparticles coated with a silicate shell were synthesized under different conditions of preparation. Our study of IR spectra showed that nanoparticles obtained from a solution of iron salts in a ratio of 2.7 : 1 had higher concentrations of OH groups on their surfaces. These samples also had a high blocking temperature, relative to a sample obtained from a solution of iron salts in a ratio of 2 : 1.

Isolating DNA from blood cells showed that the 2 : 1 sample was not inferior to the silicate sorbent in terms of the quantity and quality of the extracted DNA. The 2.7 : 1 sample was too inefficient for isolation.

Particles synthesized from a solution of iron salts in a 2 : 1 ratio appeared to be better for the magnetic separation of DNA. This was because these particles were in a ferromagnetic state with T_B near room temperature, and there were no OH groups on the surface that could inhibit the bonding of DNA molecules.

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