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Polysaccharide-coated iron oxide nanoparticles: Synthesis, properties, surface modification

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ABSTRACT

In this work, magnetite nanoparticles coated with polysaccharides were synthesized. Arabinogalactan and chitosan were used as polysaccharides. The possibilities of immobilization of biospecific molecules on the surface of the obtained composites were studied. Experiments on covalent immobilization of biospecific molecules on magnetic nanoparticles coated with a polysaccharide showed a high density of immobilized molecules. This suggests the use of such materials in bioanalytical systems or as affinity sorbents.

1. Introduction

Magnetic nanoparticles are of great interest for biology and medicine, both for basic research and applied in various fields, such as magnetic resonance imaging, magnetic separation, drug delivery, hyperthermia [1–4]. The magnetic properties of nanoparticles are determined by finite-size and surface effects, which determine the difference between nanoparticles and bulk materials.

One of the problems associated with magnetic particles is their inherent instability over a long time. Fine particles tend to form agglomerates. Various stabilizers or coating agents, in particular, various polysaccharides, are often used to prevent particle aggregation [5–7]. The chemical and structural diversity of natural polysaccharides (chain length, monosaccharide sequence, stereochemistry, etc.) provides the maximum opportunity for the development of advanced multifunctional materials for biomedicine [8]. Magnetic nanobiocomposites based on polysaccharides have the properties of both a stabilizing polysaccharide matrix and a magnetic core, as well as high sorption capacity. Due to this, they can be used as a carrier in the creation of highly specific and highly sensitive biosensors and affinity sorbents for the detection or separation of biomolecules from biological fluids, as well as in the creation of a targeted delivery system for drugs with targeted action [9].

The work is devoted to the synthesis of magnetic nanoparticles and the preparation on their basis of soluble and colloidal-stable complexes with arabinogalactan and chitosan. The possibilities of immobilization of biospecific molecules on the surface of the obtained composites by chemical synthesis were studied.

2. Experimental

2.1. Preparation and characterization of nanoparticles

Magnetite nanoparticles were prepared by co-precipitation method as follows. 10 g of iron sulfate ($FeSO_4 \cdot 7H_2O$) and 3 g of a stabilizing agent (chitosan, arabinogalactan) were dissolved in 100 mL of distilled water. At a temperature of 80 °C, a solution of sodium hydroxide NaOH (0.1 M) was added to the solution until a neutral pH was reached. Coated magnetite nanoparticles were thoroughly washed with distilled water to remove ions. Thus, magnetic nanoparticles coated with arabinogalactan (MNP ~ arabinogalactan) and chitosan (MNP ~ chitosan) were prepared.

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Samples were examined on a Hitachi HT7700 transmission electron microscope (accelerating voltage 100 kV) of the Center for Collective Use, Krasnoyarsk Scientific Center, Russian Academy of Science, Siberian Branch. IR spectra were obtained on samples in the KBr matrix in the spectral range from 350 to 4000 cm^{-1}. The static magnetic measurements were performed on an automated vibrating sample magnetometer in fields of up to 15 kOe at room temperature.

2.2. Covalent attachment of biotin to magnetic particles

MNP ~ arabinogalactan: 1 mL of 1 M sodium acetate (Sigma) (in 3 N NaOH) was added to 1 mL of the particle suspension and incubated for 2 h at room temperature (RT). After washing (with 0.1 M Bicine pH 8.5), 0.5 mL of the mixture of 1 M N-(3-dimethylamino propyl)-N'-ethylcarbodiimide hidrochloride (EDC, Sigma-Aldrich) and 1 M hexamethylenediamine dihydrochloride (Sigma-Aldrich) in 0.1 M Bicine pH 8.5 were added. After incubation (2.5 h, RT) the particles were washed with 0.1 M $NaHCO_3$ then with 20 mM Tris-HCl pH 7.0. The particles were incubated with 1 mg of biotinamidohexanoic acid N-hydroxysuccinimide ester (Bio-Su, Sigma) in 0.1 M $NaHCO_3$ overnight at 4 °C and washed with 0.1 M K/Na phosphate buffer pH 7.0.

The determination of the amount of NH_2 groups on the surface of MNP ~ chitosan was performed by titration with hydrochloric acid as described in [10].

MNP ~ chitosan: Bio-Su (twofold molar excess of to the number of NH_2 groups) was added to the 0.3 mL of the particles suspension pre-washed with 0.1 M $NaHCO_3$. The mixture was incubated overnight at 4 °C and washed with 0.1 M K/Na phosphate buffer pH 7.0.

2.3. Covalent attachment of streptavidin to magnetic particles

MNP ~ arabinogalactan: 0.5 mL of the particle suspension (in 0.1 M K/Na phosphate buffer pH 8.0) were incubated with 0.2 mL of p-benzoquinone (200 mg/mL, in 20% aqueous ethanol) for 2 h at RT and washed with 0.1 M Bicine pH 8.5. Then 1 mg of recombinant full size streptavidin in 0.1 M Bicine pH 8.5 were added to the particles, incubated overnight at 4 °C and the particles were washed with 0.1 M K/Na phosphate buffer pH 7.0.

MNP ~ chitosan: succinimidyl 4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC, Thermo Scientific) twofold molar excess to the number of NH_2 groups were added to 0.3 mL of the particles suspension pre-washed with 0.1 M $NaHCO_3$. The mixture was incubated for 2 h at RT and washed with 0.1 M K/Na phosphate buffer pH 7.0. 2 mg of streptavidin was incubated with 10-fold molar excess of 2-iminothiolane hydrochloride (Sigma) in 0.1 M Bicine pH 8.5 for 1 h at RT. Then the excess reagent removed gel chromatography on HiTrapDesalting Column (GE Healthcare) equilibrated with 0.1 M K/Na phosphate buffer pH 7.0. Iminothiolan-activated streptavidin was incubated with SMCC-activated particles overnight at 4 °C. Then the particles were washed with the same buffer.

3. Results and discussion

Fig. 1 shows the images obtained using a high-resolution transmission electron microscope of magnetite nanoparticles prepared with arabinogalactan (a) and chitosan (d) as well as the corresponding microdiffraction patterns (b, e) and particle size distribution (c, f). The shape of the particles is nanoplates with a thickness an order of magnitude smaller than the diameter. The average nanoplate diameters were 21.5 nm and 52 nm for the MNP ~ arabinogalactan sample and the MNP ~ chitosan sample, respectively. The thickness of the nanoplates was ~5 nm. Diffraction patterns of the studied samples are characteristic of spinel ferrites.

The results of magnetic studies (saturation magnetization, remanent magnetization, coercivity) of nanoparticles are shown in Table 1. Low values of coercivity indicate that particles with this

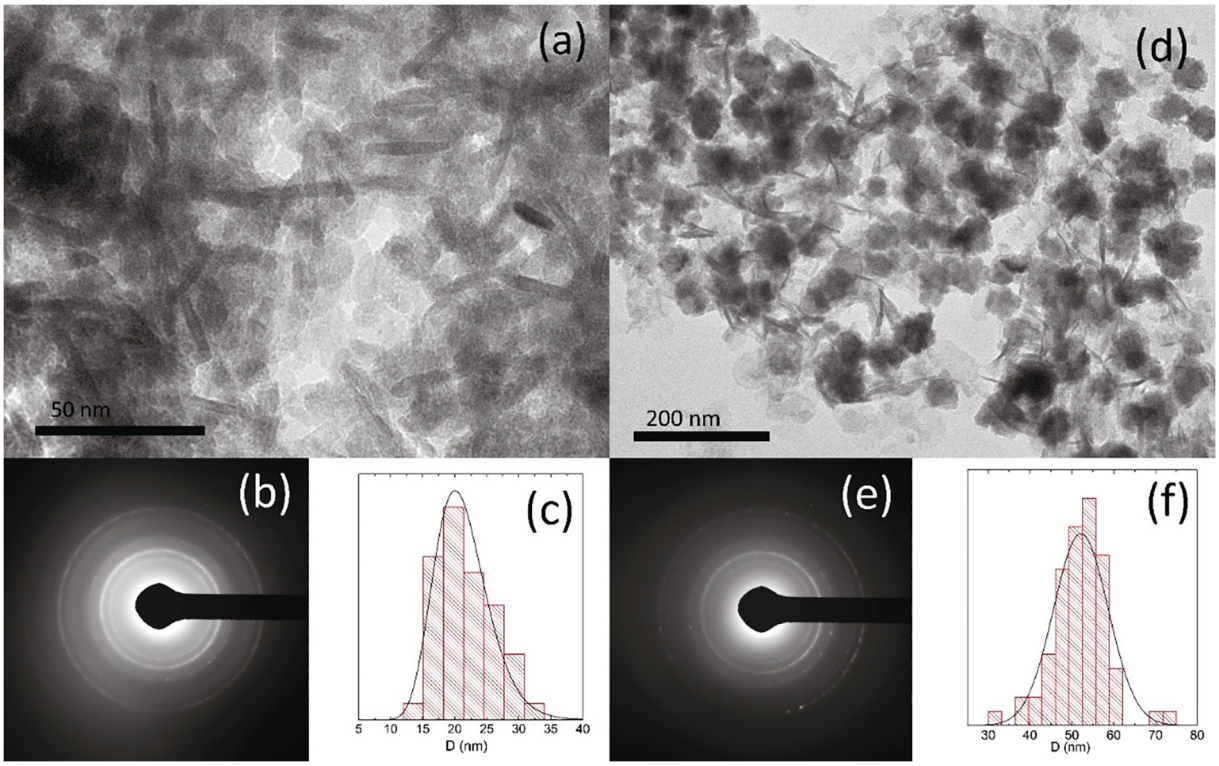

Fig. 1. TEM-images, microdiffraction patterns and particle size distribution of MNP ~ arabinogalactan (a, b, c) and MNP ~ chitosan (d, e, f).

Table 1
Magnetic hysteresis parameters.

	H_C, Oe	M_S, emu/g	M_R, emu/g
MNP ~ arabinogalactan	12.5	9.91	0.40
MNP ~ chitosan	11.5	4.15	0.13

size are close to the transition to the superparamagnetic state. The relatively small values of the saturation magnetization of nanoparticles are due to the polysaccharide shell.

After a qualitative analysis of the spectra of polysaccharide samples and nanoparticle/polysaccharide samples, the main characteristic frequencies were determined. In the spectra in Fig. 2a, the most intense are the vibrations of the hydroxyl group at $\nu = 3400\ cm^{-1}$. All hydroxyl groups are involved in hydrogen bonds since the IR absorption of free hydroxyl groups is ~3650 cm^{-1}. The band at 1370 cm^{-1} characterizes the deformation vibrations of the C–H bonds. The intense band at 1074 cm^{-1} in both spectra refers to the stretching of C–O bonds. The spectrum of the MNP ~ arabinogalactan sample differs from the spectrum of pure arabinogalactan in the 500 cm^{-1} region. An oscillation of 564 cm^{-1} relates to stretching modes of Fe-O iron oxides. By the appearance of this vibration, we can speak of the formation of a bond between the magnetic nanoparticles of iron oxides and arabinogalactan.

The spectrum of MNP ~ chitosan sample is different from the spectrum of pure chitosan, especially in the region of 500 cm^{-1}. Two intense lines appear with frequencies 395, 588 cm^{-1}, which may indicate that iron oxide is introduced into the structure of this "polysaccharides".

Covalent binding of biospecific molecules on coated magnetic nanoparticles provides strong linkages between the molecules and carriers. Biotin and streptavidin, widely used in binding analyses as a molecules forming high affinity and specific complexes, have been applied in current research as a model molecules of different size.

Biotin or streptavidin molecules were immobilized on the surface of both kind of obtained MNPs using a chain of known chemical reactions [11] (Fig. 3).

MNP ~ arabinogalactan and MNP ~ chitosan were modified using their surface hydroxyl and amino groups respectively. The amount of bound biotin or streptavidin molecules was determined colorimetrically according to Green method [12]. As a result, 1.37 nmol and 7 nmol of biotin was immobilized on the surface of 1 mg MNP ~ arabinogalactan and MNP ~ chitosan, respectively. Streptavidin is a relatively large protein (64 kDa), that creates steric barriers to its immobilization. That's why the amount of immobilized protein is smaller: 0.54 nmol and 0.9 nmol per milligram of MNP ~ arabinogalactan and MNP ~ chitosan, respectively.

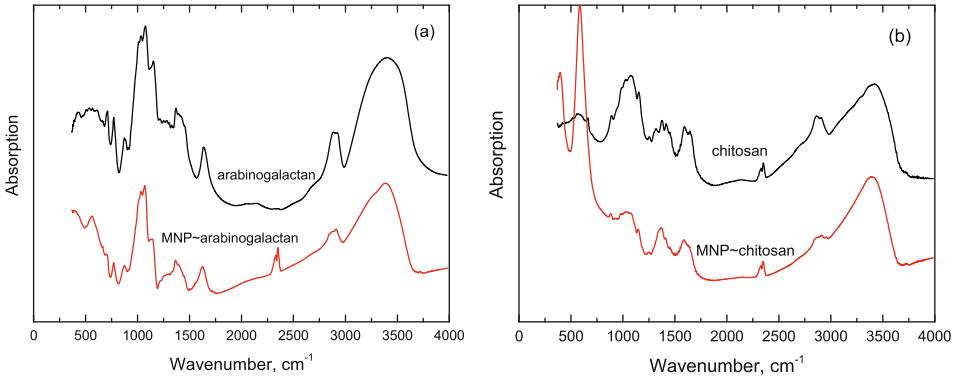

Fig. 2. FTIR spectra of MNP ~ arabinogalactan (a) and MNP ~ chitosan (b).

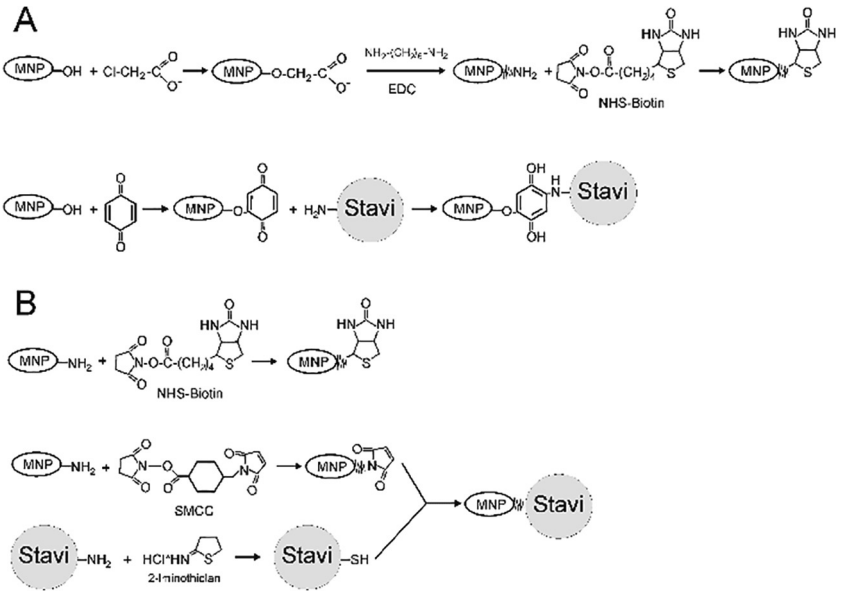

Fig. 3. Chemical immobilization of biotin and streptavidin molecules on the surface of MNP ~ arabinogalactan (A) and MNP ~ chitosan (B).

It was interesting to estimate the density of protein molecules immobilized on the MNP. For calculation the diameter value as 20 and 50 nm (Fig. 1) and particle content per 1 mg of the samples of 8.7×10^{13} and 7.4×10^{12} for MNP ~ arabinogalactan and MNP ~ chitosan, respectively were used. It was calculated that as many as 3×10^{15} and 0.93×10^{16} of streptavidin molecules were bound per m^2 of MNP ~ arabinogalactan and MNP ~ chitosan, respectively. It is close to the immunoglobulin density adsorbed on the surface of commercial microplate for immunoassay – 1.9×10^{16} molecules per m^2 (Corning, USA).

Thus, primary experiments on the covalent immobilization of biospecific molecules on carbohydrate-bearing magnetic nanoparticles show the availability of the corresponding surface functional groups for chemical modification. The obtained samples are characterized by a high density of immobilized molecules, which determines the prospects of creating biospecific nanoparticles based on these materials for use in bioanalytical systems or as affinity sorbents.

CRediT authorship contribution statement

S.V. Stolyar: Supervision, Writing - review & editing, Conceptualization. **V.V. Krasitskaya:** Writing - original draft, Investigation. **L.A. Frank:** Conceptualization, Investigation. **R.N. Yaroslavtsev:** Writing - original draft, Investigation. **L.A. Chekanova:** Investigation. **Y.V. Gerasimova:** Investigation. **M.N. Volochaev:** Investigation. **M.Sh. Bairmani:** Investigation. **D.A. Velikanov:** Investigation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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