ISSN 1063-7842, Technical Physics, 2020, Vol. 65, No. 9, pp. 1377–1383. © Pleiades Publishing, Ltd., 2020.
Russian Text © The Author(s), 2020, published in Zhurnal Tekhnicheskoi Fiziki, 2020, Vol. 90, No. 9, pp. 1435–1441.

NANOMATERIALS IN BIOLOGY AND MEDICINE

Fabrication of Cylindrical Magnetic Nanoparticles for Functionalization of Polyelectrolyte Microcapsules

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Received December 16, 2019; revised December 16, 2019; accepted February 17, 2020

Abstract—A problem of fabrication of magnetic polymer capsules for targeted drug delivery is considered. Magnetic nanoparticles are used in the problem under study. A method for fabrication of such particles involving synthesis of layered nanowires with alternating layers of the specified magnetic metal and sacrificial layers of nonmagnetic metal is proposed. A method for template-assisted synthesis based on ac-potential electroplating of specified metals in the pores of track membranes is used to fabricate wires with a diameter of 100 nm with nickel layers of 400 and 200 nm. A method for the subsequent extraction of nickel fragments using selective etching (removal) of copper fragments is developed. Procedures that prevent aggregation of magnetic nanoparticles and penetration of the nanoparticles in the shells of polymer capsules are considered.

DOI: 10.1134/S1063784220090121

INTRODUCTION

It is known that magnetic particles are widely employed in biology and medicine. Targeted drug delivery is among topical problems under study. For efficient treatment, a drug must be delivered to organism at a specified location and be activated therein. (Relatively high doses are needed when the organism is affected as a whole, and side effects are probable.) A method to solve such a problem can be based on encapsulation, which employs deposition of a drug to a capsule that is used for targeted delivery. The corresponding works were actively performed over the last 10–15 years.

Successful applications of polymer multilayer capsules for drug delivery and procedure for controlled release can be found in [1]. Analysis of several methods for fabrication of capsules, delivery of drug molecules into the capsules and to the capsule shells, and possible methods for functionalization have been presented in [2]. Application of polyelectrolyte and nanocomposite microcapsules (fabricated with the aid of layer deposition) for encapsulation and drug delivery for diagnostic purposes has been reviewed in [3]. Several methods to improve the functional properties of capsules (e.g., ultrasound, external magnetic fields (MFs), and laser and short-wavelength irradiation) have been considered. It has been mentioned that short-term ultrasonic or IR irradiation can be used for opening of capsules.

A method for functionalization of capsules can be based on introduction of magnetic particles into the capsules. In this case, delivery of capsules to the desired location can be performed with the aid of MF. (Note that such an approach does not solve several problem: in particular, the propagation of capsules is primarily determined by the blood flow, so that field correction can be difficult to implement. The method is efficient for vessels with relatively low blood flow.) The method has been studied in several works. In particular, polyelectrolyte capsules have been modified for magnetic delivery using electrostatic adsorption of preliminary synthesized magnetite nanoparticles on the charged part of the shell [4]. In an alternative approach of [5–7], magnetic nanoparticles have been synthesized directly on the capsule shell.

Magnetic particles introduced in a polymer capsule can be used to solve the problem of local heating. Such a problem emerges when targeted thermal action on a diseased organ (or cells of certain type, for example, cancer cells) is required.

The problem is solved using effect of ac MF on capsules with magnetic particles that were preliminary delivered to the required location. MF with a certain frequency leads to heating of such particles due to conversion of field energy into thermal energy. Note that

the above heating (hyperthermia) can be supplemented with controlled release of encapsulated drug [8, 9]. The efficiency of the MF-energy conversion into thermal energy can be increased using nonspherical (e.g., cubic) nanoparticles introduced into the capsule shell [10].

Another problem that can be solved with the aid of magnetic particles introduced into a capsule with drug is the activation of such a capsule. In particular, a pulsed MF of 0.18 T has been used in [11] for effect on magnetic colloidosomes. The resulting iterative compression has led to liberation of encapsulated substance. Such a procedure provides stable release controlled by compression and relaxation cycles of colloidosomes but the capsules with a diameter of up to 500 μm are too large for drug delivery in vivo. AC MF is a universal external effect that provides opening of magnetic microcapsules in biomedical applications. Human body tissues are transparent for such an effect and are not damaged. Effect of low-frequency MF on the capsule at the desired location leads to rotational or vibrational motion of magnetic particles in the capsule shell and conversion of the MF energy into mechanic energy. The motion of particles leads to local or complete destruction of the capsule shell and drug release. Such a problem has been solved with the aid of magnetic (e.g., magnetite [12]) nanoparticles with almost spherical shapes that are evidently nonoptimal. Several additional problems must be solved for fabrication of magnetic particles for biomedical applications. In particular, a protecting layer may be needed on the surface of a magnetic nanoparticle. Electrodeposition has been used to fabricate iron nanowires (NWs) with a length of 15 μm (polycrystalline) or 1 μm (single-crystalline) in the pores of porous aluminum oxide with a diameter of 50 nm [13]. Further oxidation (in a template at a temperature of 150°C and time ranging from 10 min to 72 h) has been used for fabrication of the F_3O_4 oxide layer on the surface. Such an oxide layer prevents further oxidation of NWs at room temperature. The resulting "core–shell" structures have been studied using methods of magnetometry and magnetic force microscopy. It has been shown that the magnetic properties strongly depend on the conditions for preparation and shell thickness (the saturation magnetization and residual magnetization decrease with increasing thickness). Note also that the NWs with oxide shells exhibit higher biocompatibility.

A general review of the application of magnetic particles in therapy and diagnostics and the corresponding problems have been reviewed in [14]. It has been shown that the energy effect of MF on a biochemical system can be enhanced using magnetic nanoparticles (MNPs) that serve as mediators of the field effect. While inside organism, MNPs become primary targets for the MF effect providing high sensitivity of the system to external MFs. MNPs provide, first, enhancement (by several orders of magnitude) of the MF effect and, second, localization of such an effect in the desired region (provided that the MNPs are delivered to this region). Thus, the total intensity of the external action can be decreased and the effect can be localized. For such purposes, MNPs are functionalized using one or several shells containing the specified bioactive substance (ligand shells). Normally, the radius of such a functionalized particle is up to 100 nm. In addition, such shells protect MNPs against oxidation and provide the required hydrophobic properties that prevent aggregation and diminish cytotoxicity. Such MNPs with ligand shells can be supplemented with the capsules that contain a bioactive substance in the central part surrounded with shells containing magnetic particles (the radius of such nanocapsules is up to 100–300 nm). In the presence of external field, magnetomechanical activation of MNPs takes place: depending on the field (dc–ac and uniform–nonuniform), the MNPs may exhibit rotational, translational, rovibrational, or translational–vibrational motion. Such motion may lead to local heat liberation or destruction of the capsule. Evidently, such an effect depends on the parameters of the external magnetic field.

In the above scenarios, particles have uncontrolled (normally, almost spherical) shapes. In this work, we propose functionalization of polymer capsules using elongated magnetic nanosized particles (cylindrical MNPs (CMNPs)). Application of such particles with a predetermined aspect ratio and controlled magnetic moment makes it possible to solve the problems of both targeted drug delivery and controlled opening of polymer capsules due to mechanical damage (destruction). Such damage can be due to rotation of magnetic particles (located inside the capsule shell) in the presence of ac magnetic field with a certain frequency. In this work, we use nanosized fragments of NWs as magnetic particles. The approach is based on fabrication of heterostructure NPs consisting of layers of magnetic metal (Ni) with the specified length that are spaced by Cu metal layers, which must be removed afterwards (sacrificial layers). Such NPs can be obtained with the aid of template-assisted synthesis based on electrochemical filling of track membranes. The principles of the membrane-based synthesis can be found in [15]. Fabrication of layered NPs (variant of the above structures) has been described in several works. State of the art, in particular, specific features of fabrication of layered NPs has been presented in monograph [16]. A method for fragmentation of the layered NPs has been proposed in [17]. However, the fragmentation has been performed for NPs preliminary fixed on the surface for alternative purposes. We have discussed several particular problems of fabrication of long NWs consisting of nickel and copper layers and control of the layer thickness in [18]. The process has been performed in electrolyte containing ions of both metals. Alternation of metal layers has been implemented using periodic changes of the growth voltage. After

removal of the growth polymer template, the samples have been studied using SEM and TEM methods that have shown alternation of layers with different compositions. The task of this work is to develop a method for fragmentation of such NWs using selective etching. The final task is to obtain calibrated (with respect to size) elongated CMNPs and study such particles included in the shells of multilayer polyelectrolyte microcapsules.

1. EXPERIMENTAL

1.1. Materials and Methods

Track membranes from Joint Institute for Nuclear Research were used as growth matrices. The thickness of the polyethylene terephthalate (PETP) membrane is 12 μm, the pore diameter is 100 nm, and the pore density 1.2×10^9 cm^{-2}. For electrodeposition, a thin conducting copper layer is sputtered on one side of the matrix with the aid of thermal vacuum sputtering (VUP-4). Then, the sputtered layer is galvanically fixed ($CuSO_4 \cdot 5H_2O$, 200 g/L and H_2SO_4, 10 g/L) to form a continuous conducting layer that serves as a working electrode. For electrodeposition, we use the following composition of electrolyte: $NiSO_4 \cdot 7H_2O$, 0.7 M; $CuSO_4 \cdot 5H_2O$, 0.025 M; and H_3BO_3, 0.51 M (where M is mol/L). A galvanic cell with an area of the deposited sample of 2.5 cm^2 has been constructed at the Design Bureau of the Crystallography and Photonics Research Center. An Elins P-2X potentiostat–galvanostat served as the current source for electrodeposition. The device allows time recording of the galvanic process.

For fabrication of polymer microcapsules (PMs), we use the following materials and reagents: calcium chloride $CaCl_2$, sodium carbonate Na_2CO_3 from Acros Organics, polyallylamine hydrochloride (PAH), sodium chloride NaCl, trisodium salt of ethylenediamine tetraacetic acid (EDTA), polysorbate 80, and tannin acid from Sigma–Aldrich. In the experiments, we use the deionized water obtained with the aid of the Millipore Milli-Q Plus device.

The sizes and morphology of the CMNPs and PMs were studied with the aid of SEM using the JCM-6000plus device equipped with a unit for elemental analysis and JSM- 7401F device (JEOL, Japan). Both devices worked in the regime of secondary electrons.

The zeta potentials on the PM surface in suspension were measured using a Malvern Zetasizer Nano ZS computerized analyzer at a temperature of 25°C.

1.2. Experimental Results

NWs were layer-by-layer electrodeposited in the pores of the track membranes. The electrodeposition involves deposition of nickel layers (magnetic layers to be extracted as single CMNPs) and copper (sacrificial) layers. Such an approach allows synthesis of multiple CMNPs in a single pore channel. Sequential deposition of different metals was implemented using sequential changes of the potential in the course of growth. The potentials for copper and nickel layers are 0.7 and 1.8 V, respectively [18].

Fig. 1. SEM image of the suspension of layered NPs.

The SEM method was used to monitor the formation of the heterostructure NWs. Prior to the measurements, the NWs were separated from the growth polymer matrix and, then, NWs were separated from the copper substrate using an ultrasonic bath. Note that preliminary experiments were performed for NWs consisting of copper and cobalt layers, since such a combination of metals makes it possible to obtain relatively high contrast in microscopic analysis. Figure 1 shows an example of the SEM image obtained for a suspension of the layered NWs.

The SEM image clearly shows single layers. We also analyzed different growth regimes. In the best regime with respect to exact control of the thickness of deposited layers, the potential depends on the charge flow (such a study has been started in [19]). Two regimes were used: the thicknesses of the nickel layers were 200 and 400 nm, respectively, and the thickness of copper layers was 100 nm in both cases. The numbers of the deposition periods were 10 and 5, respectively. After the electrodeposition, the matrix was removed in concentrated alkali NaOH at a temperature of 60°C and a time of 2 h. The task of the next stage was the fragmentation of the layered NWs. The problem was solved with the aid of removal of sacrificial layers and copper substrate using selective etching. In this work, we tested four etching agents. The first one is the solution of NH_4OH (150 g/L) in water with $CuSO_4 \cdot 7H_2O$ (1 g/L). Such a solution dissolves copper and does not react with nickel. The main disadvantage is a relatively long etching time (about 72 h at room temperature). To accelerate the process, we used a solution of citric acid (300 g/L) and NaCl (50 g/L) in H_2O_2. A disadvantage of the method is related to violent reaction with copper and slower reaction with

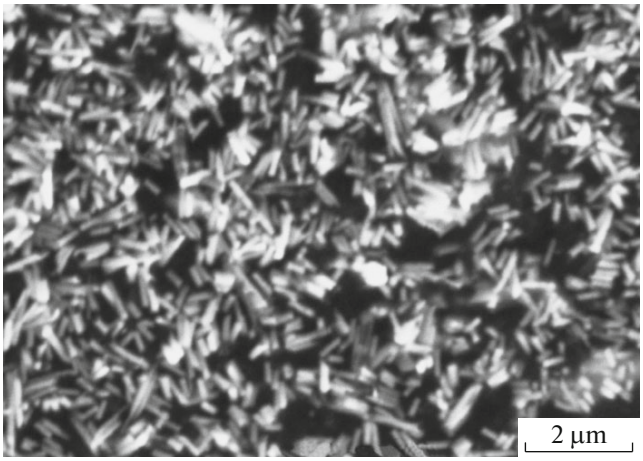

Fig. 2. SEM image of agglomerates of nickel CMNPs.

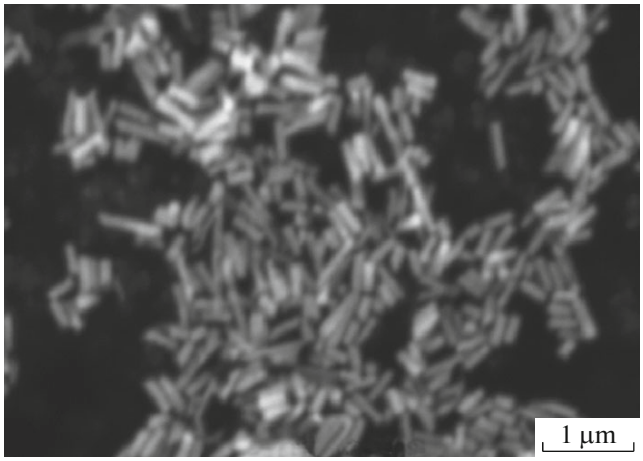

Fig. 3. SEM image of the modified CMNPs.

nickel. It is impossible to determine the moment at which copper is completely dissolved and critical damage of the nickel layer is absent. For a decrease in the etching rate and activity of the etching agent, we diluted the solution with water by factors of 2 and 4. For the two-fold dilution, the process was slower but the reaction with the functional part (nickel layer) took place. The best results were obtained for the four-fold dilution. In the absence of the reaction with the functional part of the NW, the time of copper dissolution was about 1 h. Thus, the optimal variant of the selective etching agent for copper in the presence of nickel is as follows: H_2O_2 (250 g/L), $(HOOCCH_2)_2C(OH)COOH$ (75 g/L), and NaCl (12.5 g/L).

1.2.1. Electron microscopy of the CMNP suspension

The CMNP suspension was studied with the aid of SEM methods. Figure 2 shows the SEM image of the extracted particles.

Analysis of the images yields identical sizes of particles corresponding to the specified regime of electrodeposition. Note also that most particles aggregate, and such an undesired process must be minimized.

1.2.2. Modification of the CMNP surface

To suppress the aggregation, we modify the CMNP surface using charged molecules. For this purpose, we add a solution of polysorbate 80 or tannin acid with a concentration of 2 mg/mL to the aqueous suspension of the particles, incubate on a shaker for 15 min, separate the modified particles from the aqueous phase, and store them as aqueous suspension. Figure 3 shows the modified CMNPs. The proposed method makes it possible to suppress the aggregation of particles.

The purpose of the next stage is to introduce CMNPs into the shell of polyelectrolyte capsules (PECs).

1.2.3. Fabrication of microcapsules with CMNPs

Colloid particles of calcium carbonate were produced using mass crystallization in accordance with the reaction $CaCl_2 + Na_2CO_3 = CaCO_3 + 2NaCl$. The known method was used for fabrication of micrometer-size particles: equivalent volumes of the $CaCl_2$ and Na_2CO_3 solutions with a concentration of 0.1 M were stirred in a vessel with a magnetic stirrer at a rate of 500 rpm for 30 s at a temperature of 25°C. After stirring, the suspension of spherical microparticles was stored for 5–7 min to finish the crystallization of calcium carbonate. Then, the $CaCO_3$ particles were thrice washed with deionized water to remove Na^+ and Cl^- ions. Layer-by-layer adsorption of oppositely charged macromolecules was used to form the polyelectrolyte shell on the $CaCO_3$ particles. The shell consists of a two electrolytes: poly(allyl amine hydrochloride) (PAC) and polystyrene sulfonate (PSS). Calcium carbonate microparticles have a small negative surface charge, so that positive polyelectrolyte (PAC) is deposited as the first layer. PAC solution (1 mL) in 0.15 M NaCl was added to the $CaCO_3$ particles (0.015 g). The suspension was stirred for 15 min using a compact stirrer and centrifuged for 3 min. Then, the supernatant was used and the particles were thrice washed with water. The same procedure was performed using 1 mL of the polyanion (PSS) solution in 0.15 M NaCl. To prevent aggregation of particles in the course of deposition of the first two layers, the tubes with the suspension were placed for 10 s in an Elma Elmasonic S10H ultrasonic bath at a frequency of 35 kHz. The NPs under study have a small negative charge, so that the particles are introduced into the shell using adsorption on a layer of a positively charged

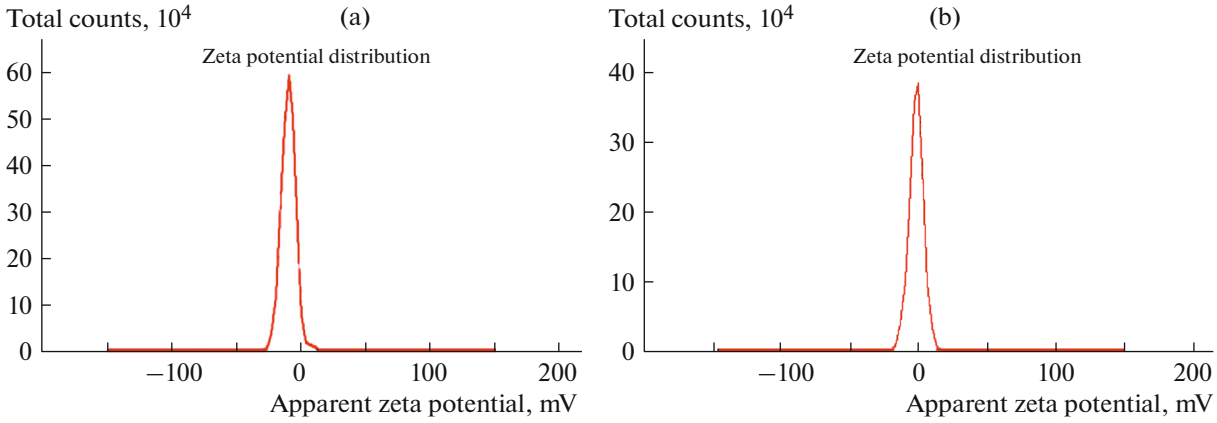

Fig. 4. Zeta potential of CMNPs with lengths of (a) 200 and (b) 400 nm.

electrolyte. In this work, we fabricated capsules with the following composition of the shells: PAC/PSS/PAC/NP/PSS/PAC/PSS. After deposition of the desired number of layers 0.2 M EDTA solution with pH 7.5 was added to the suspension for dissolving of the $CaCO_3$ particles. After 15-min-long incubation with EDTA, the capsules were thrice washed with water. The resulting capsules were stored as an aqueous suspension at a temperature of 4°C.

The CMNP were incorporated into the shells of polymer capsules with the aid of electrostatic adsorption. For optimization of the procedure, we measured the zeta potential, which appeared to be -8 ± 2 and -4 ± 1 mV for CMNPs with lengths of 200 and 400 nm, respectively (Fig. 4). With allowance for the negative surface charge, we performed adsorption of CMNPs on a layer of positively charged electrolyte.

Table 1 presents the zeta potentials for the $CaCO_3$ particles with polyelectrolyte layers. Switching of the charge polarity due to alternation of polyanion and polycation characterizes the layer-by-layer formation of the capsules. When the CMNP layer is deposited, the zeta potential changes sign, which indicates efficient adsorption of particles on the PAC layer. Samples of capsules with CMNP lengths of 200 and 400 nm have equal (within experimental error) zeta potentials. Such a result proves the absence of the effect of NP sizes on the stability of capsules.

The SEM study proves the presence of CMNPs in the capsule shells (Fig. 5).

The SEM images show that the CMNPs are incorporated in the polymer shell, which was the purpose of this work. Note nonuniform distribution of particles, presumably, due to aggregation. To further suppress the aggregation and improve the surface properties, the CMNPs were coated with surfactants. The analysis of the SEM images of the capsules formed with application of modified CMNPs shows that the surface modification slightly suppresses the aggregation of particles and does not affect the efficiency of the CMNP incorporation into the polyelectrolyte shell.

Further optimization of the CMNP incorporation in the shell will be based on a search for more efficient methods for suppression of strong aggregation including passivation and temporal transition to nonmagnetic state (e.g., due to heating).

Table 1. Changes of zeta potential upon formation of polyelectrolyte capsules

Sequence of layer deposition	Zeta potential, mV	
$CaCO_3$	0 ± 1	
$CaCO_3$/PAC	3 ± 1	
$CaCO_3$/PAC/PSS	-22 ± 2	
$CaCO_3$/PAC/PSS/PAC	7 ± 2	
$CaCO_3$/PAC/PSS/PAC/NP	NP 200 nm	NP 400 nm
	-24 ± 2	-12 ± 1
$CaCO_3$/PAC/PSS/PAC/NP/PAC/PSS/PAC	6 ± 2	5 ± 1
PAC/PSS/PAC/NP/PAC/PSS/PAC capsules after dissolution of $CaCO_3$	8 ± 2	7 ± 1

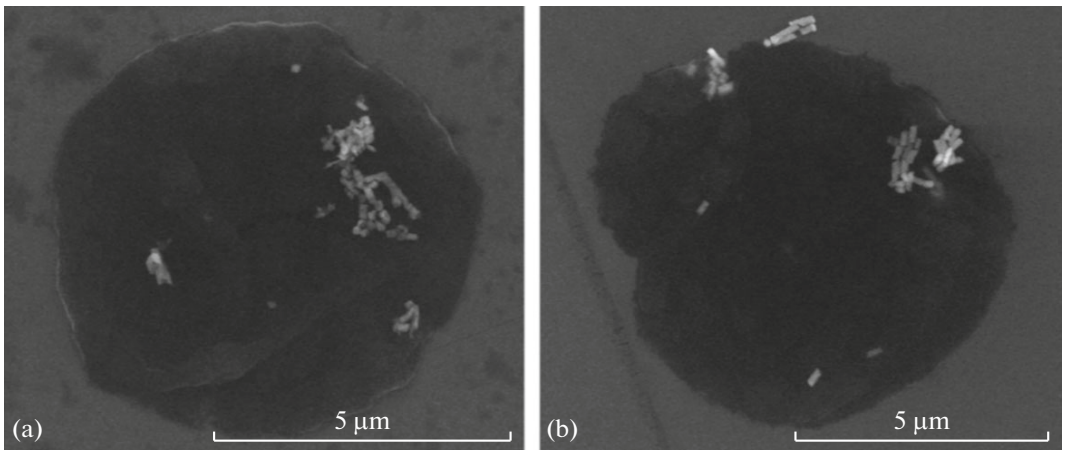

Fig. 5. SEM images of polymer microcapsules (PAC/PSS)3 with CMNPs with lengths of (a) 200 and (b) 400 nm in a shell of polymer capsule.

CONCLUSIONS

We have proposed a method for fabrication of cylindrical magnetic nanoparticles from layered nanowires using template-assisted synthesis in the pores of track membranes. Experiments with NPs consisting of alternating copper and nickel layers show that magnetic particles (nickel cylinders) can be separated using selective etching of sacrificial layers of the second metal (copper). We have proposed a method for processing of such particles (passivation) that makes it possible to diminish aggregation. It has been shown that NPs with lengths of 200 and 400 nm can be incorporated in the (PAC/PSS)3 microcapsules.

ACKNOWLEDGMENTS

We are grateful to P.Yu. Apel' for providing track membranes and R. Kamyshinskii for the SEM study of the capsules.

FUNDING

This work was supported by the Ministry of Science and Higher Education of the Russian Federation in the framework of the State Contract using Core Facility of the Crystallography and Photonics Research Center. The synthesis and characterization of magnetic nanowires were supported by the Ministry of Education and Science of the Russian Federation (project RFMEFI62119X0035). Fabrication of the magnetized capsules was supported in part by the Russian Foundation for Basic Research (project no. 18-53-34007 "Kuba_t").

CONFLICT OF INTERESTS

The authors declare that they have no conflicts of interest.

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Translated by A. Chikishev