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Multifunctional biocompatible coatings on magnetic nanoparticles

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Abstract. Methods for coating formation on magnetic nanoparticles used in biology and medicine are considered. Key requirements to the coatings are formulated, namely, biocompatibility, stability, the possibility of attachment of pharmaceutical agents, and the absence of toxicity. The behaviour of nanoparticle/coating nanosystems in the body including penetration through cellular membranes and the excretion rates and routes is analyzed. Parameters characterizing the magnetic properties of these systems and their magnetic controllability are described. Factors limiting the applications of magnetically controlled nanosystems for targeted drug delivery are discussed. The bibliography includes 405 references.

I. Introduction

Nanoparticles and composite materials based on them exhibit unique electrical, chemical and magnetic properties and therefore attract considerable attention of researchers. Numerous studies and reviews are concerned with prospects for their application in various fields of science and technology.[1 – 10]

One of the most promising research avenues involves utilization of magnetic nanoparticles (MNPs) in biology and medicine, namely, they are used in immunoassay for purification of biological fluids, for hyperthermia, drug and gene delivery, for separation of cells and molecules, as well as in tissue engineering.[11 – 20] The advantage of MNPs consists in magnetic controllability. Solving most problems mentioned above requires the design of nanosystems containing not only MNPs, but also appropriate coating-forming materials of biological or synthetic nature. Coatings determine the nature of the interaction of nanosystems with biological fluids in the body and should be non-toxic, biocompatible and ensure localization of nanosystems in particular tissues, organs or cells. Coatings on MNPs can be functionalized by biovectors (antibodies, enzymes, nucleotides, *etc*.) for targeting particular biological systems (or recognition of these systems) by drugs or genes. The nature of coatings determines the excretion rate of particles from the body, the residence time of nanosystems in the body, the distribution of particles in organs and tissues and the diffusion rates of nanosystems and drugs in tissues. Coating should ensure stability of nanosystems, *i.e*., preclude their agglomeration and not be desorbed from the surface of particles.

Immobilization of coatings on the surface of MNPs is a complicated problem. In biological media, the MNPs/coating nanosystems interact with biological fluids, proteins, cell membranes, organelles, DNA and other biological objects; as a result, the continuity of mobile coating can be violated and certain parameters of the surface of the nanosystem will change. The chemical composition of the surface of nanosystems determines their behaviour and the nature of interaction in biological media. Interactions at the nanosystem/biological medium interface proceed by complex mechanisms and are a subject of special studies.[21 – 24]

In this review we present a comprehensive analysis of problems posed when fabricating biocompatible coatings based on organic and inorganic substances for biomedical applications on MNPs. We will compare the available methods for fabrication of various types of coatings from different materials, consider the advantages and drawbacks

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of coatings, the behaviour of nanosystems in dispersions and assess prospects for their use in biomedicine. We will briefly outline some characteristics of the magnetic properties of MNPs and describe the influence of the magnetic properties and size of MNPs on the ability to control them using external magnetic fields (magnetic controllability). Also, key features of the behaviour of nanosystems in the body are discussed. Published data on such important processes as transmembrane transport of magnetic nanosystems and their excretion from the body are analyzed. Prospects for biomedical applications of nanosystems and factors precluding progress in the technology of targeted drug transport using magnetically controlled carriers are also considered.

Problems concerning the synthesis and structure of nanoparticles are left out of consideration because these topics are adequately addressed in the literature.[7, 9, 12, 14, 25–31]

II. Main characteristics of magnetic particles

Magnetic properties were reported for a variety of nanoparticles including γ-Fe_2O_3 (maghemite), Fe_3O_4 (magnetite), $MgFe_2O_4$, $MnFe_2O_4$, FePt, CoPt, $NiFe_2O_4$, NiO, $NiFe_2O_4$, Co_3O_4, $CoFe_2O_4$, Mn_3O_4, MnO_2, Fe, Co, Ni, *etc.*[7, 9, 12, 14, 25–31] Detailed information on the magnetic properties of small particles can be found in the literature.[29, 32, 33] In this Section we will consider those magnetic properties that are important for biomedical applications of MNPs. The magnetic properties of nanoparticles depend on their chemical composition, particle size,[34] shape, and morphology.[35] They can also be influenced by certain characteristics of coatings.[36, 37] In most cases, a necessaty step in the design of nanosystems for biology and medicine is to ensure stability of MNP dispersions.

1. Magnetization, single-domain state and superparamagnetism

A key parameter of the magnetic state of macroscopic and nanosized objects is the magnetization† (M), *i.e.*, the magnetic moment (μ) per unit volume (V)[38]

$$M = \frac{\mu}{V}.$$

The magnetic state is also characterized by the magnetic induction (B) and the magnetic field strength (H); these parameters are interrelated by

$$B = H + 4\pi M.$$

The saturation magnetization (M_s) of bulk materials is first of all determined by the nature of the material and the temperature of the environment. For instance, the magnetization of nickel is 490 emu cm^{-3} at 77 K (Ref. 39) and 486 emu cm^{-3} at 298 K (Ref. 40); the magnetization of iron in the vicinity of 0 K is 1752 emu cm^{-3} (*cf.* 1714 emu cm^{-3} at room temperature[40]); the magnetization of cadmium is 2640 emu cm^{-3} at 77 K.[39] The saturation magnetization of nanoparticles is usually lower than that of a bulk sample and decreases with the MNP size.[41–43] This effect can be explained by the difference between the magnetic properties of the surface layer of a nanoparticle and its core,[43] and by the oxidation process on the particle surface.[41, 42]

Bulk magnetic materials have a multidomain structure composed of uniformly magnetized domains separated by domain walls (Fig. 1).[44] The formation of domain walls is associated with the balance between the magnetostatic energy (E_{ms}), which increases in proportion to the sample volume, and the formation energy of domain walls (E_{dw}), which is proportional to the surface area of the domain wall. By reducing the sample volume one can attain such a particle size at which the domain wall energy will be higher than the magnetostatic energy responsible for the magnetic state of the sample. Small particles can be single-domain and exhibit the properties of superparamagnetics. The critical size (D_c) corresponding to the single-domain state can be as large as tens and hundreds nanometres depending on the nature of the material.[32, 45]

Material	Fe	Ni	Co	Fe_3O_4	γ-Fe_2O_3	$SmCo_5$
D_c/nm	15	55	70	128	166	750

The magnetic moment of a single-domain particle is proportional to its volume.

Multidomain and single-domain particles can remagnetize in external magnetic fields (see Fig. 1). Remagnetization of multidomain particles is a result of the motion of domain walls; the main remagnetization mechanism of single-domain particles is associated with coherent rotation of individual atomic magnetic moments (Bohr magnetons).[46] Remagnetization of single-domain particles is precluded by the magnetic anisotropy, which is proportional to the magnetic anisotropy constant and the sample volume.[47]

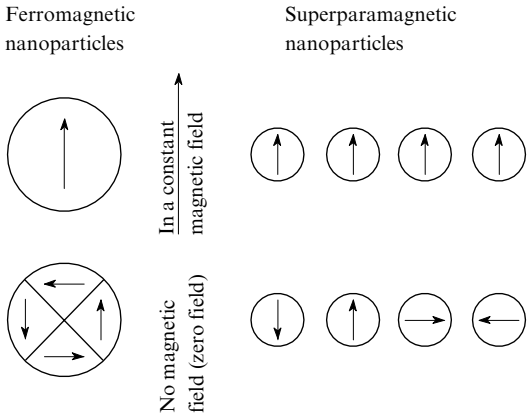


Figure 1. Behaviour of particles in zero and a nonzero magnetic field.
In zero magnetic field, ferromagnetic particles exhibit a residual magnetization, whereas superparamagnetic particles show no residual magnetization owing to fast rotation of the magnetic moments. In a constant magnetic field, the domain walls disappear and all magnetic moments (Bohr magnetons) are aligned to the magnetic lines of force. The magnetic moments of single-domain superparamagnetic particles are aligned along the magnetic force lines.

† The magnetization is measured in A m^{-1} in the SI system and in erg G^{-1} cm^{-3} in the CGS system; 1 erg G^{-1} cm^{-3} = 1 G = 10^3 A m^{-1}. The following units: emu cm^{-3} or emu g^{-1} are often used.

The energy of magnetic anisotropy (magnetic energy) of a nanoparticle depends on the direction of its magnetization vector relative to crystallographic axes. The magnetic energy increases with increasing angle between the magnetization vector and the easy magnetization axis (Fig. 2). This axis is the direction of the vector along which the energy is minimum.[48] The anisotropy energy is the product of the nanoparticle volume by the magnetocrystalline anisotropy constant (K_a)

$$E_a = K_a V.$$

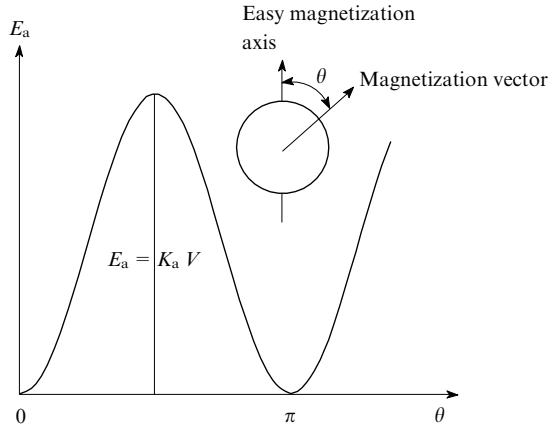


Figure 2. Magnetic anisotropy energy (E_a) plotted *vs.* angle (θ) between the magnetization vector and the easy magnetization axis of MNP.

In addition to the magnetocrystalline anisotropy, which determines the direction of the easy magnetization axis, there are two parameters responsible for the direction of the magnetization vector of a particle. These are the shape anisotropy (K_{sh}), which appears under the action of demagnetizing fields in the sample, and the surface anisotropy (K_s). For spherical samples, the demagnetizing fields are equal to zero, but they increase as the system elongates (in this case, $K_{sh} > 0$). Owing to small sizes of nanoparticles, their magnetic properties depend strongly on the surface state, *viz.*, the surface anisotropy.[49, 50] The surface anisotropy is due to the crystal lattice symmetry violation. Interactions of various compounds with the nanoparticle surface cause changes in the electronic environment of particles[37] and can lead to changes in the oxidation state of constituent atoms.[51] The magnetic properties of nanoparticles are influenced by both organic and inorganic coatings. When varying the nanoparticle size and shape or in the presence of surface defects, changes in the volume ratio of the MNP core, its surface layer and the coatings affect the magnetic anisotropy and magnetization.

The influence of various coatings on the geometry, electronic and magnetic properties of cobalt nanoparticles less than 10 nm in diameter was analyzed.[52] It was found that in the case of gold coatings, the magnetic anisotropy of cobalt MNPs decreased and that of iron MNPs increased compared to bulk cobalt and iron samples, respectively.[53] Also, the opposite influence of cobalt and gold coatings were reported:[26] the magnetization increased for cobalt-sheathed iron oxide MNPs and decreased for carbon-, gold- and silver-sheathed iron oxide MNPs. This would be associated with the magnetic properties of cobalt; however, the magnetization of iron oxide MNPs coated with platinum and palladium also increased. The thicker the coating the stronger its influence on the magnetic properties of MNPs.[54]

Consider the phenomenon of superparamagnetism. In the case of strong magnetic anisotropy, the magnetic moment vector is aligned to the easy magnetization axis. However, thermal fluctuations can cause jumps of the magnetic moment vector from one easy magnetization axis to another (so-called Néel relaxation).[55] As the particle size decreases, the magnetic moment vector jumps become more intense. The kinetics of such jumps (Néel relaxation time) can be described by the equation[56]

$$\tau_N = \tau_0(E_a) \exp \frac{E_a}{kT},$$

where $\tau_0(E_a)$ is the pre-exponent which depends on the anisotropy energy.

At $E_a \ll kT$, the exponential term tends to unity and the Néel relaxation time is determined by the pre-exponential term, which decreases as the anisotropy energy increases. At $E_a \gg kT$, the Néel relaxation time is governed by the exponent, which predicts a fast increase in the relaxation time with increasing E_a.

If the Néel relaxation time is longer than the magnetization measuring time, the magnetization curve is irreversible and a hysteresis loop appears. In the case of strong anisotropy, the relaxation time can be as long as a few hundred years; therefore, such materials can be utilized for fabricating data storage systems (hard disks).

Solving medical problems requires the use of particles with short relaxation times and that the thermal energy kT be high enough to overcome the anisotropy barrier $K_a V$; also, the magnetic moment vector of nanoparticles should jump with ease from one easy magnetization axis to another. Such systems behave as paramagnets; they are called superparamagnets. The magnetization curves of superparamagnets exhibit no hysteresis loops and the relaxation time of the magnetic moment is about 10^{-9} s.[57]

It should be noted that nanoparticle colloidal solutions (dispersions) rather than nanoparticle powders are most often used in biology and medicine. Therefore, the equilibrium state is established through both the Néel relaxation mechanism and rotation of particles (Brownian relaxation) and the total relaxation rate ($1/\tau$) is determined by the sum (Fig. 3)

$$\frac{1}{\tau} = \frac{1}{\tau_N} + \frac{1}{\tau_B},$$

where τ is the total relaxation time, τ_N is the Néel relaxation time; τ_B is the Brownian relaxation time

$$\tau_B = \frac{3V\eta}{kT}.$$

η is the viscosity.

For large particles, the Brownian relaxation time is shorter than the Néel relaxation time because the Brownian

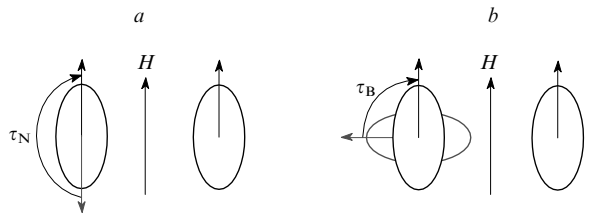


Figure 3. Néel relaxation (*a*) and Brownian relaxation (*b*) of a particle in a magnetic fluid.

relaxation is proportional to the particle volume, the Néel relaxation time being an exponential function of the particle volume. Contrary to this, the Néel relaxation time of small particles is much shorter than the corresponding Brownian relaxation time.

Thus, the magnetic properties of MNPs are to a great extent determined by their nature and particle size. The key parameters dependent on the particle size include the saturation magnetization and the relaxation time.

2. Stability of magnetic colloidal systems. Aggregation of nanoparticles in external magnetic field

Particles in magnetic dispersions are small enough, which makes it possible to avoid their sedimentation under the action of gravity force. However, van der Waals and magnetic interactions between particles will cause their agglomeration. A number of approaches was developed to preclude this process. One of them includes the use of surfactants that create a molecular 'cloud' on the surface of particles, which counteracts van der Waals forces.[58] Yet another widely used stabilization technique involves creation of an eletrical double layer on the surface of nanoparticles. Ionic groups form a charged diffuse layer on the surface, which ensures electrostatic repulsion of particles.[59,60] This method of stabilization is applicable in the case of polar liquid media, *e.g.*, water, containing necessary concentrations of counter-ions. For instance, the surface of iron oxide MNPs can be charged positively or negatively depending on the pH value of the medium because the surface iron atoms of such MNPs behave as Lewis acids and coordinate molecules with lone electron pairs. Owing to fast dissociation of water the surface of iron oxide particles is modified with amphoteric hydroxyl groups, which can react with acids and bases[61]

$$\text{surface FeOH} + \text{H}^+ \rightleftharpoons \text{surface FeOH}_2^+,$$

$$\text{surface FeOH} + \text{HO}^- \rightleftharpoons \text{surface FeO}^- + \text{H}_2\text{O}.$$

Stabilization is based on the appearance of obstacles to the approach of particles to distances at which the attractive energy is higher than the energy of thermal motion.[62] In particualr, stability of colloidal systems is maintained through balance between three types of interactions, namely, attractive van der Waals (E_W) and magnetic dipole–dipole (E_{dd}) interactions and electrostatic repulsion (E_{el}). Therefore, the total potential energy (E_t) of the system can be expressed as:

$$E_t = E_{el} + E_W + E_{dd}.$$

The last term should be included in this expression in the case of induced or permanent magnetic moments.

Single-domain particles can be treated as dipoles with the magnetic moment proportional to the particle volume

$$\mu = M_s V.$$

The energy of magnetic interactions between two particles with the magnetic moments μ_1 and μ_2 placed at the centre-to-centre distance r_{12} is given by[63]

$$E_{dd} = \frac{\mu_0}{4\pi r_{12}^3}\left[\vec{\mu}_1\vec{\mu}_2 - 3(\vec{\mu}_1 r_{12})(\vec{\mu}_2 r_{12})\right].$$

The energetically most favourable configuration of the particle implies aligned magnetic moments ('head-to-tail' configuration).

In stable colloidal systems, the particle radius usually does not exceed 10–15 nm. As the particle size increases, the probability of agglomeration increases because the magnetic moment increases or the condition for single-domain state is violated. Such particles are of interest due to specific features of their behaviour in magnetic fields. A magnetic field aligns the magnetic moments of MNPs and favours the formation of anisotropic structures comprised of nanoparticles.[64,65]

Magnetic colloids are composed of identical ferromagnetic particles suspended in nonmagnetic fluids. To characterize the dipole–dipole interaction leading to agglomeration, the pairing constant

$$\lambda = \frac{\mu^2}{r_{12}^3 kT}$$

was introduced.[66] It characterizes the system in zero external magnetic field. At $\lambda > \lambda_c > 4$ (λ_c is the critical pairing constant), magnetic particles can form aggregates at $H = 0$.[67] In magnetic fluids, where the volume fraction of particles (φ) is at most 10^{-2}, particles are about 10 nm in diameter, the surface stabilizing layer is 2 nm thick and the dipole pairing parameter is less than unity. Such colloidal systems remain stable in zero external magnetic field and the dipole–dipole interaction between particles can be neglected. Stevens and Grest[68] established that λ_c decreases with increasing external magnetic field strength which thus favours aggregation of magnetic particles.

Numerical simulation of the behaviour of nanoparticles in colloidal systems showed that in the case of strong dipole–dipole interaction and small volume fraction of particles, they form chain aggregates and that the number and length of the aggregates are described by increasing functions of the external magnetic field strength.[69,70] The magnetization and the initial magnetic susceptibility of long chains thus formed appeared to be higher than the corresponding parameters of non-aggregated magnetic particles.[71] The presence of aggregates also influences the diffusion and hydrodynamic properties of magnetic fluids.[72,73]

The formation of linear chain aggregates in magnetic fluids can be accompanied by side (lateral) aggregation of a number of aligned chains. A number of models were proposed to explain this behaviour of chains.[74,75]

Experimental studies of aggregation were carried out by optical[76] and diffraction methods.[77] Magnetic fluids

become optically anisotropic[78,79] and their viscosity abruptly increases in external magnetic fields.[80] In some cases, experimental results were in qualitative agreement with the conclusions drawn in theoretical studies.[74,75]

In addition to optical and diffraction methods, linear aggregates of magnetic particles are studied by ferromagnetic[81–83] and electron paramagnetic resonance.[84,85] Magnetic anisotropy and inter-particle interaction cause the resonance conditions and, consequently, the parameters of ferromagnetic resonance lines to change.

III. Biomedical applications of MNPs

The properties of nanoparticles are determined by the particle size, chemical composition and shape which can vary depending on the synthesis conditions. The choice of a particular type of MNPs for biomedical applications depends first of all on the problems to be solved.

It should be noted that cobalt, nickel and Nd–Fe–B alloy MNPs exhibit strong magnetic properties but can be readily oxidized and are toxic for humans,[86] whereas iron oxide (both magnetite and maghemite) MNPs show a minimum toxicity and are thought to be best candidates for biomedical applications.[87,88] An undisputable advantage of magnetic particles is the possibility of controlling their motion using an external constant magnetic field and to heat them by an oscillating magnetic field. Design of nanosystems containing not only MNPs, but also biological or synthetic coating-forming materials functionalized with biovectors (proteins, enzymes, antibodies, nucleotides, *etc.*) and treatment medications is the most rapidly progressing field of bionanotechnologies. Biovectors (binding sites) provide recognition of targets (tissues, specified cell types) and drug delivery to cells.[89,90]

Magnetic nanoparticles can be used for diagnosis (magnetic resonance imaging)[91,92] and therapy (hyperthermia; drug and gene delivery to malignant tissues and cells);[93,94] therefore, magnetic controllability of MNPs is of great importance. The concept of magnetically guided delivery involves transfer of magnetic particles to a particular region using a localized magnetic field gradient, keeping until completion of therapeutic intervention and subsequent excretion. Using MNPs, one can perform *in vitro* separation of cells, proteins and other biomolecules through specific recognition.[95–97] Separation technologies allow one to separate genetically damaged DNA/RNA molecules,[98,99] extract cancer stem cells by recognition of surface antigenes,[100] and purify biological fluids.[12] Magnetic nanoparticles can be used to analyze changes in cells due to the action of various factors[101] including the influence of drugs and hormones on the mechanical properties of membranes and the cell response.

Successful use of nanoparticles in biomedicine depends on some parameters. Often, the necessary factor is the superparamagnetic behaviour and high magnetization of particles. Magnetic nanoparticles can penetrate cell membranes, their narrow size distribution facilitates correct calculations of dosages and administration procedures. In this connection, it is not surprising that modern fundamental studies are aimed to synthesize uniform MNPs, give them the biocompatibility and functional properties necessary for the delivery to the target regions in the body and to determine the subsequent fate of particles in blood flow. The design and fabrication of stable biocompatible coatings on MNPs are among the key aspects in the design of nanosystems for biomedical applications.

1. Drug and gene delivery

In 1960, Freeman *et al.*[102] proposed that magnetic particles can move in the vascular system in the body and accumulate in a specified region under the action of external magnetic field. Before the studies on the vector delivery of drugs began, it had been proposed to utilize magnetic particles in antiangiogenic therapy.[103] The idea of magnetically guided drug delivery for treatment of malignant neoplasms using magnetic microspheres was proposed in the late 1970s.[104] In 1983, therapeutic action of doxorubicin on magnetically guided albumin microspheres intravenously administered in rats was demonstrated.[105] In 1994, biodegradable polylactic acid microspheres containing magnetite and the ^{90}Y radioactive isotope were prepared and successfully used in the treatment of subdermal tumour.[106] Magnetic nanoparticles were for the first time used for drug delivery in the 1990s taking epidoxorubicin as an example.[107] Probably, low efficiency of the drug delivery systems based on MNPs was due to the fact that they were rapidly recognized by macrophages and then excreted from the body.[108] Nonmagnetic liposomal drug delivery systems were the first to be used in clinical trials and then certified.[17,109] Attractive features of magnetic targeting of drugs include the possibility to localize drugs in the body and to accumulate them in tumours.[110] Also, special coatings can provide prolonged drug release.[6]

Gene (nucleic acid) delivery methods using magnetically guided nanoparticles have been developed since the early 21st century.[111,112] Good results were obtained in preclinical studies.[113–115] Theoretically, by introducing genes into the target cells it is possible to correct genetic errors and stimulate the immune system.[116] Gene delivery to cells under the action of magnetic field,[112] or magnetofection, combines the physical and chemical approaches.[117,118] To additionally increase the transfection efficiency, MNPs can be bound to viruses[118,119] and nucleic acids.[120]

Successful use of vector drug and gene delivery is possible if nanosystems exhibit the following properties: narrow size distribution, stability and biocompatibility of coatings, sufficiently long *in vivo* residence time, the possibility of specific targeting and high transfection efficiency.

2. Magnetic resonance imaging

Magnetic resonance studies began in the 1970s. This is the most efficient noninvasive method in clinical medicine, which makes it possible to determine the internal structure and functions of organs.[121]

Magnetic resonance imaging is based on the phenomenon of nuclear magnetic resonance (NMR), mainly involving protons of water molecules. A signal contrast mainly depends on the proton spin density and on the longitudinal (spin–lattice relaxation) and transverse (spin–spin relaxation) relaxation times denoted by T_1 and T_2, respectively. The relaxation times can be shortened by introducing NMR contrast agents into the system. Such contrast agents have magnetic moments (spins) and create local magnetic fields. Magnetic nanoparticles also belong to NMR contrast agents. The relaxation times change as a result of the interaction of the proton spin with the unpaired electron spin. If this interaction occurs when a water molecule and the contrast agent molecule contact each other, the spin–lattice relaxation time changes. In this case, one deals with

the inner-sphere relaxation mechanism. When a water proton is at some distance from the contrast agent, it 'senses' the local field of the contrast agent; this leads to shortening of the spin–spin relaxation time. This is the case for the outer-sphere relaxation mechanism. Magnetic nanoparticles used as NMR contrast agents mainly cause the spin–spin relaxation time to shorten. Changes in the relaxation times are detected as blackening of corresponding regions. The effect of the NMR contrast agent on the relaxation time can be characterized by the so-called relaxivity

$$R_{1,2} = \frac{1}{T_{1,2}}.$$

The larger the $R_{1,2}$ value the more pronounced the contrast effect. Based on the model for inner- and outer-sphere relaxation,[122] one can predict that the relaxivity will change in the presence of magnetic particles.[123] As MNPs arrive at damaged tissues, the contrast between the normal and damaged tissues increases.[25]

Magnetic nanoparticles are used for both diagnosis (to detect invaded tissue in the early stages of disease)[124,125] and for *in vivo*/*in vitro* studies of biochemical processes.[126] They can also be used in magnetic resonance imaging for simultaneous diagnosis and therapy.[127] As a rule, NMR contrast agents are based on iron oxide nanoparticles; iron, cobalt and nickel particles are used much more rarely.[128–131] Surface modification of MNPs allows one to use them as contrast agents for magnetic resonance studies of various organs and tissues.[131–134]

To analyze different types of cells, MNPs are conjugated to peptides, antibodies, as well as fragments of protein and other biovector molecules.[90] Detailed information on the design of targetable nanosystems for cell and molecule studies was reported in the reviews.[14,15] At present, a large number of researchers work in the field of design of contrast agents based on MNPs and systems for cell and molecule studies.[131,135–137] The existing NMR contrast agents based on iron oxide MNPs are mainly aimed to be used in organ-specific visualization of liver.[138]

A number of contrast agents based on iron oxides with superparamagnetic properties (Feridex/Endorem, Resovist/Cliavist, Ferumoxtran-10, Clariscan, Lumirem/Gastromark) were reviewed.[139] Some of them are commercially available in the USA, Japan and European countries.

Summing up, as in the case of development of nanosystems for vector drug delivery, the efficiency of magnetic resonance imaging is determined by the totality of the properties of nanoparticles, stability and biocompatibility of coatings, the ability of nanosystems to avoid the action of reticuloendothelial system (see below) and accumulate in the target.

3. Hyperthermia and thermoablation

There are two methods of cancer treatment by heating of MNPs, *viz*., hyperthermia and thermoablation. Hyperthermia involves a local thermal action. The idea of utilizing magnetic hyperthermia is based on heat release from superparamagnetic particles upon absorption of the energy of an oscillating magnetic field. Alternating magnetic field induces electric currents in metallic objects (especially in those characterized by co-operative magnetic behaviour). The magnitude of the current is proportional to the magnetic field amplitude and to the size of the object. Depending on the particle size and shape and on the properties of tissue, the operating frequency should be in the range from 100 to 1000 kHz.[140] Reorientation of the magnetization vectors in particles (in the course of rotation of single-domain particles and displacement of domain walls in large particles) is accompanied by Brownian motion of MNPs in suspensions.[18]

Hyperthermia is used in biology and medicine, first of all, in therapy of cancer. A number of studies on application of magnetic particles in hyperthermia were reported.[140–145] The heating intensity depends on the particle size and shape and on the properties of tissue determining the ability of MNPs to localization. The first communication in this field was reported in 1993.[146] Most studies on the choice of particles for hyperthermia are based on calculations. In particular, it was found that optimum heating of tissues is attained with 14-nm magnetite MNPs with a narrow size distribution.[147] Also, it was established that the MNPs with the Curie temperature in the range 40–60 °C are most appropriate for hyperthermia.[148]

Some types of cancer cells are sensitive to temperatures above 41 °C (*cf*. 46 °C for normal cells).[149,150] For instance, at high temperatures, receptors on the surface of cancer cells become more recognizable to normal killer cells.[151] Selective distant action of alternating magnetic field on cancer cells *in vitro* was reported.[149] It was also reported that, when using hyperthermia, the heating temperature can reach a value of 47 °C.[152]

The possibility of use of *in vivo* hyperthermia of human cancer was demonstrated.[153]

Thermoablation involves heating to higher temperatures (up to 55 °C) but its action range is not localized in tumours.[20] The need for utilizing thermoablation is due to tumour recurrence after hyperthermia treatment. Thermoablation of a tumour is possible is an electromagnetic wave is emitted by a high-frequency source (>100 MHz) implanted into the tumour or using a number of external microwave sources.[154]

Thus, the use of MNPs in hyperthermia and thermoablation treatment requires control of (i) the particle size and shape during the synthesis and (ii) the nature and thickness of coatings on these particles.

IV. Adsorption on MNPs and clustering processes

Adsorption of molecules is the most important step in the design of coatings on the surface of MNPs. Fabrication of biocompatible coatings requires knowledge of the regularities of adsorption of low-molecular-mass molecules, synthetic polymers and biomacromolecules on nanoparticles. However, in spite of considerable research efforts in this field, adsorption on the surface of nanoparticles is still poorly studied. Coating of nanoparticles also requires taking into account competing processes, namely, the possibility of replacement of the adsorption layer molecules by some molecules from the environment.

Main parameters affecting the strength and type of interactions between adsorbates and adsorbent particle include the nanoparticle size, the surface properties of nanoparticles (hydrophilicity, charge, chemical composition), the nature of adsorbate molecules (molecular mass, conformation, functional groups, charge, hydrophilicity, *etc*.).

In most cases, nanoparticles based on iron oxides are used for biomedical applications (see above). The surface of such particles is modified using physical and chemical adsorption of various substances through hydrophobic interactions, as well as electrostatic and covalent interactions with hydroxyl groups.[155] Surfactants and polymers containing carboxyl, hydroxyl, phosphate, phosphonate, sulfate and sulfonate groups can be physisorbed on the surface of iron oxide MNPs and form chemical bonds with it.[7, 156, 157] By analogy with corresponding coordination compounds the molecules containing electron-donor atoms (O, N, P, S) involved in the bonds with the surface of MNPs are called ligands.[158]

Dopamine can be bound to the surface of iron oxide MNPs through the formation of a rigid ring (Fig. 4 *a*).[159, 160] Ascorbic acid is also octahedrally coordinated by iron atoms on the surface of MNPs (Fig. 4 *b*).[161] Adsorption of citric acid on the surface of magnetite MNPs occurs through coordination of one or two (depending on the steric effects and the surface curvature of MNPs) carboxyl groups.[162] Maghemite MNPs were coated with an anionic coating made of *meso*-2,3-disulfanylsuccinic acid (DMSA) containing two carboxyl groups.[163] Magnetic nanoparticles are stabilized in dispersions using surfactants containing carboxyl groups (lauric and oleic acids).[164] The acids form dense coatings on MNPs. However, such bonds of acids with the surface of MNPs can be readily broken on heating or upon exchange with a carboxylic acid of some other composition (Fig. 4 *c*).

The strength of the bond between compounds containing hydroxyl groups and the surface of MNPs depends on the number of hydroxyl groups.[165] The use of polyols in the synthesis of MNPs allowed one to utilize the interaction between polyols and the surface of MNP seeds for control of seeding and growth of particles and to synthesise monodisperse particles.[166, 167]

Desorption of molecules from the surface of MNPs is initiated by, *e.g.*, heating, dilution, change in pH, presence of compounds that are more prone to adsorption (competing processes). For instance, desorption of dextran macromolecules from the surface of iron oxide MNPs occurs upon dilution of the dispersion,[165] while oleic acid can be displaced by ethanol.[168] It was established that the desorption temperatures of alkylphosphonates and alkyl phosphates are higher than those of carboxylates.[164]

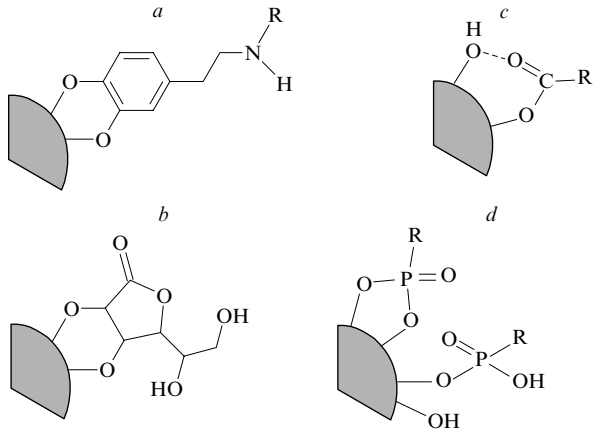


Figure 4. Adsorption of dopamine derivatives (*a*), ascorbic acid (*b*), carboxylic acids (*c*) and phosphonates (*d*) on the surface of MNPs containing hydroxyl groups.

Phosphonic acids $RPO(OH)_2$ and phosphates have higher affinities to the surfaces of maghemite, magnetite and ferrite ($MnFe_2O_4$, $NiFe_2O_4$, $CoFe_2O_4$, $ZnFe_2O_3$) MNPs[169, 170] and form Fe–O–P bonds with the surface of the magnetite MNPs.[164, 171] These bonds are stronger than those formed by carboxyl groups, but their density on the surface of MNPs is lower.[168] The pH values less than 5 are favourable for adsorption of orthophosphate anion (PO_4^{3-}) on the surface of magnetite MNPs 40 nm in diameter.[172] Phosphate fragments can be used to stabilize magnetite hydrosols at low pH values. Bonds formed by phosphonates and dopamine with the surface of MNPs remain unbroken in a wider range of pH values.

The binding mechanism of sulfonic and phosphonic acids with the surface of iron oxide MNPs was studied.[157, 173] Two schemes of binding the phosphonate group to the surface were proposed, through one or two oxygen atoms of the functional group (Fig. 4 *d*).

A widely used method of surface modification of MNPs is based on the chemical interaction between the surface hydroxyl groups and organosilicon compounds. The creation of silica coatings on magnetic particles can be accompanied by the formation of clusters comprising two or more MNPs coated with a common shell.[174]

Adsorption of dextran molecules on the surface of iron oxide MNPs mainly occurs through hydrogen bonds and chelation.[175] It should be noted that in some cases adsorption of macromolecules on MNPs is accompanied by clustering of MNPs owing to simultaneous adsorption of macromolecules on the surfaces of different particles. The larger the charge of the macromolecule and the number of constituent functional groups the higher the clustering probability. For instance, a cationic polymer polyethyleneimine (PEI)[176, 177] is adsorbed on the surface of magnetite MNPs in a wide range of pH values of the medium (see Ref. 14) with the formation of clusters.[178] Also, PEI can displace other polymers, *e.g.*, polyvinylpyrrolidone (PVP) and polyvinyl alcohol (PVA) from the surface of MNPs. Probably, the reason for high affinity of PEI to the surface of iron oxide and ferrite MNPs consists in the ability of PVP to form complexes with iron.[179] In addition to PVP and PVA, poly(ethylene glycol) (PEG) is also adsorbed on the surface iron oxide MNPs. Usually, PEG is adsorbed on individual particles and no clustering occurs.[180]

Coating of MNPs with starch is accompanied by the formation of clusters 200–300 nm in size.[181] It should be noted that magnetic controllability of clusters is better than that of individual core/shell particles (nanosystems).

In biological fluids, proteins are adsorbed on nanoparticles and nanosystems.[182] Adsorption of proteins can influence the stability and disturb the functionality of the coating as well as favour excretion of particles from the body. Also, the protein functionality can be disturbed. It is mainly albumin that is adsorbed upon an increase in the hydrophilicity of the nanoparticle surface in blood flow.[183] If the MNP surface contains nonpolar groups, adsorption of hydrophobic or amphiphilic compounds occurs. An increase in the charge of particles and a decrease in the hydrophilicity of their surface lead to an increase in the adsorption capacity of nanosystems compared to blood

plasma proteins.[184] The charge of polymers forming the surface layer of nanoparticles affects their stability in biological fluids, because negatively charged MNPs bind to proteins with an isoelectric point >5.5 (immunoglobulin G), while positively charged MNPs bind to proteins with an isoelectric point <5.5 (albumin).[185] An ideal polymeric coating should preclude adsorption of proteins and other compounds in biological fluids. In particular, nanosystems coated with PEG,[184,186] poloxamers and poloxamines[187] are characterized by reduced adsorption of proteins and long residence time in blood flow.

When nanoparticles are introduced into the body, the number of protein molecules adsorbed on them depends on the affinity of the protein molecules to the MNP surface and on the local concentrations of nanoparticles and proteins in the biological medium. The surfaces of iron oxide MNPs and MNPs coated with polymers can interact with albumin, immunoglobulins, complement components, fibrinogen and lipoproteins.[21] For instance, the surface of nanoparticles in the blood flow can be initially covered with albumin and fibrinogen which are then displaced by proteins with the higher affinity and slower adsorption kinetics.[182] If the content of the high-affinity protein in biological fluids (intraocular fluid, tracheo-bronchial secretion) is low, the surface will be covered with high concentrations of low-affinity proteins (*e.g.*, albumin).

Adsorption of two blood plasma proteins, albumin and fibrinogen, on the surface of magnetite MNPs was analyzed (Table 1).[188,189] It was established that fibrinogen has a higher affinity to the surface of magnetite MNPs compared to albumin and is adsorbed with the formation of clusters (>1000 nm in size). Also, a spin-label EPR study[192] showed that fibrinogen can displace serum albumin molecules from the surface of magnetite MNPs.

V. Methods of introduction of nanosystems into the body and their subsequent fate

1. Methods of introduction of nanosystems into the body. Magnetic controllability

The potential of nanosystems for diagnosis and therapy is due to the possibility of choosing the target and ensure precise targeting. Targeted drug delivery allows one to reduce drug doses and accumulation of drugs outside the target regions, and, consequently, to minimize side effects.[193] The efficiency of drug delivery depends on the properties of nanosystems, on the method of administration and physiological conditions, namely, the size of nanosystems, characteristics of their surfaces (the ability to adsorb proteins, hydrophobicity, *etc.*), stability, concentration of particles, drug–surface bond strength, drug release kinetics, toxicity, mechanisms of excretion of particle components from the body, duration and rate of administration procedure, mass of the body, blood volume, functional state of heart and circulation time.[94,194] Precise drug delivery to malignant tissues using MNPs can be achieved using passive and active strategies, namely, with and without applying a magnetic field.[195]

Passive (uncontrolled) targeting of nanosystems utilizes the pressure gradient between vessels and tissues. High vessel perforation in tumour tissues favours accumulation of nanoparticles in them (this effect is known as superior penetration and retention) and allows one to use passive targeting of nanosystems mainly for diagnosis and therapy of cancer.[196] The residence time of nanosystems in blood flow is limited by their surface properties and by the size of the nanosystems.[197] Small particles (less than 20 nm in size) take a shorter time to be delivered to tumours but they can be faster excreted from the body. Prolonged circulation of nanosystems smaller than 500 nm in size improves their accumulation in the invaded regions.[155] It was assumed that passive targeting is possible for nanosystems whose size ranges from 30 to a few hundred nanometres.[21]

Active targeting is based on biological recognition of specific molecules (biovectors) immobilized on the surface of particles by antigens or cell receptors. The list of possible biovectors includes low-molecular-mass compounds (folic acid,[198] thiamine,[199] biotin, avidin, streptavidin, neutravidin, peptides or peptidomimetics[200,201]), proteins (transferrin, antibodies, lectins, enzymes) and their fragments,[202,203] aptamers,[204,205] carbohydrates,[206] hormones, nucleic acids, *etc.*[12,15,54,90,193,207] Biovectors can favour the overcoming of biological barriers, *e.g.*, the

Table 1. Adsorption (A) of natural and synthetic polymers.

Nanosystem	D /nm	Adsorptive	pH	A /mg g^{-1}	D* /nm	Clustering	Ref.
Fe_2O_3/DMSA	35	BSA	7.4 (4 °C)	–	46	N.O.	163
Fe_2O_3/DMSA/BSA	41	IgG	7.4 (4 °C)	–	62	N.O.	163
Fe_2O_3/SiO_2/PEG6000	50–350	BSA	7.4	see [a]	–	–	174
Fe_2O_3/SiO_2/PEG750	50–350	BSA	7.4	see [b]	–	–	174
$Fe_3O_4/C_6H_8O_7$	68	PEI	see [c,d]	–	165	+	190
$Fe_3O_4/(HPO_4^{2-},C_6H_8O_7)$	12	BSA	8.5	<600	32–45	N.O.	189
$Fe_3O_4/(HPO_4^{2-},C_6H_8O_7)$	12	FG	8.5	2540	>470	+	188
$Fe_3O_4/(HPO_4^{2-},C_6H_8O_7)$	12	PEI	7.3 [d]	2160	>1000	+	178
$Fe_3O_4/(HPO_4^{2-},C_6H_8O_7)$	12	EPEI	7.3 [d]	1980	25	N.O.	178
Fe_3O_4/chitosan	10–20	BSA	4	110	–	–	191

Note. Notations are as follows: D is the diameter of particles, D* is the diameter of particles with adsorbed shell, BSA is bovine serum albumin, IgG is immunoglobulin G, FG is fibrinogen, EPEI is ethoxylated polyethyleneimine; 'N.O.' stands for 'clustering was not observed'; 'plus' sign stands for 'clustering occurred'; and '—' means the parameter was not determined.

[a] Uptake is 3 times lower than for Fe_2O_3/SiO_2/PEG750 particles. [b] Uptake is 3 times higher than for Fe_2O_3/SiO_2/PEG6000 particles. [c] Distilled water. [d] The presence of even small amounts of PEI in solution leads to pronounced alkalinization of the medium.

blood–brain barrier.[208] Active targeting can also occur using functional groups on the surface of nanosystems.[15]

In zero magnetic field, nanosystems are delivered to targets by diffusion. Higher efficiency of drug delivery can be achieved only under the action of external factors, *e.g.*, magnetic field.[108, 194, 209] Accumulation of particles under the action of magnetic forces increases the therapeutical effect for both passive and active targeting. Also, particles can be accumulated in a specified region. Pioneering studies on targeted transportation were carried out more than four decades ago.[104] *In vivo* targeting of nanosystems in a magnetic field requires taking into account their magnetic controllability which depends on the size of nanosystems, the fraction of MNPs, the magnetic properties of MNPs, the surface properties of nanosystems and by the magnetic field parameters. The size and surface properties of nanosystems provide the possibility for particles to penetrate blood capillary walls towards the surrounding tissues (nanosystems should be at most 400–600 nm large)[193, 210–214] and determined the circulation time of particles in the body.‡

In a magnetic field, an individual ferromagnetic particle experiences forces dependent on the particle size, magnetic field strength and magnetic field gradient.[216–218] Permanent magnets, which are used in practice, are characterized by the magnetic field $H = 0.5–1$ T and the magnetic field gradient $H/w \approx 0.5$ T cm^{-1}, where w depends on the geometric sizes of the permanent magnet. For magnetic particles 400–600 nm in diameter, the forces produced by such magnets are no greater than a few tens of pN. For instance, the magnetic force produced by a cylindrical permanent magnet 5 mm in diameter ($H = 1$ T) is about 40 pN for a 500-nm particle.[219] Magnetic forces increase in proportion to the cube of the particle size.[220] Therefore, even a minor increase in size can improve the magnetic controllability of particles; however, this reduces their penetrating ability and significantly shortens the residence time of particles in the body.

In a constant magnetic field, a MNP introduced in a fluid experiences various forces, namely, the magnetic force, viscous force (Stokes force), gravity force, thermal motion forces (Brownian forces), particle–fluid interaction forces and the magnetic dipole–dipole inter-particle forces. The motion of magnetic micro- and nanoparticles is mainly influenced by viscous forces and magnetic field.[16, 221] Therefore, the key problem to be solved in the case of vector drug delivery is to choose the magnetic field parameters necessary to hold nanoparticles and counteract blood flow. It is of great importance to know how deep are blood capillaries and invaded regions and how long is the distance that can be travelled by particles moving from blood capillaries towards the surrounding tissues.[222]

When simulating the distribution profile of particles introduced into circulatory system and held together by the magnetic field, three types of the behaviour of the particles are considered. They depend on the ratio of the magnetic forces to viscous forces and are as follows:

— if, at sufficiently high blood flow rate, viscous forces much exceed the magnetic forces, particles are moved by blood flow from the administration site and spread over the body;

— if magnetic forces dominate, particles are removed using the magnet;

— if both effects compete, a boundary layer is formed at the capillary/tissue interface.

Complication of the model by taking into account blood pulsatility, agglomeration of particles, curved vessel geometry and spatial variations of magnetic field does not qualitatively change the three types of the *in vivo* behaviour of particles, but can noticeably shift the boundaries between them.[223]

A number of methods for calculations of the *in vivo* behaviour of magnetic particles were developed[224–226] because a simplified analysis is often insufficient. For instance, a magnetic field of 0.5 T (permanent magnet 5 cm long and 0.5 cm thick) was used in experiments with rats to concentrate iron oxide particles 250 nm in diameter. Even for particles placed at a depth to 1 mm (this is comparable with the skin thickness), the magnetic forces should be less than 1×10^{-13} N. (For comparison, the viscous force acting on the same particle at a minimum flow rate in the capillary of 0.1 mm s^{-1} is seven times larger.) In this case, no accumulation of particles should be observed; however, magnetic resonance data and visual control revealed that this did occur.[223] Note that simulation should be performed taking into account specific features of the action of forces, *e.g.*, the fact that particles in the vicinity of capillary walls experience weaker viscous forces; therefore, these particles can be held by a weaker magnetic field.[227] Aggregation of particles can occur in spite of surface modification.[16, 193] This effect favours an increase in the magnetic force which increases with the volume much faster than the viscous drag proportional to the diameter of particles, *i.e.*, the magnetic force necessary to hold particles increases. Calculations of the trajectory of motion of 50-nm and 10-mm magnetite particles in the magnetic field of a quadrupole magnet (magnetic characteristics 37 MGs Œ) in a viscous environment were reported.[228] Microparticles were captured by the magnetic field in 3 ms to travel a distance of 1.4 cm; the time taken by nanoparticles to travel the same distance was 16 s.

Even in the simplest case (a single straight vessel), calculations of the behaviour of magnetic fluids represent a complicated task that requires experimental verification.

The method of administration of nanosystems (intravenous, local into tumour, oral) determines their distribution in the body, the residence time in circulatory system and the possibility to reach the target. Various administration routes of nanosystems have been reviewed.[115, 193]

2. Nanosystems: penetration through cell membranes

Under particular conditions, localization of nanosystems on the cell surface is accompanied by their trapping (endocytosis). This phenomenon is of interest because practical implementation of efficient drug and gene delivery to cells requires knowledge of the trapping regularities. It should be noted that endocytosis is not the sole penetration mechanism of nanosystems into cells, *e.g.*, a mechanical breakdown of cell membrane by nanoparticles is also possible. Trapping of a nanosystem is favoured by the hydrophobicity and positive charge of its surface, by some structural

‡ The residence time of particles should be long enough to complete all tasks. Excretion of large particles by the system of mononuclear phagocytes occurs faster; according to clinical trials,[194, 215] the residence time of 100-nm Chemicell particles ('Chemicell GmbH', Germany) in blood plasma approached 30 min.

features, by the formation of a chemical bond between the biovectors (if exist) on the surface of the nanosystem and cell receptors, by the small size of the nanosystem and by energy release upon contact with cell.[229–231]

The surface of a cell, a membrane, is an elastic lipid bilayer up to 10 nm thick.[232] Stretching of the membrane counteracts trapping of the nanosystem. Endocytosis includes an inward buckling of the membrane with the formation of a pit and subsequent penetration of the nanosystem into the cell. A detailed description of the endocytosis mechanisms can be found in the literature.[21,230,233] Nanosystems delivered to the cell are split by special enzymes. The larger the nanosystem the larger the surface area of contact with the membrane surface and, consequently, the higher the energy released upon trapping. There is a threshold size of nanosystems such that no endocytosis of smaller systems occurs. As the size of the nanosystem increases considerably, trapping by the cell proceeds slower.[234] The thickness, the density and the shape of the coating also influence the kinetics of trapping by the cell.[229,230] At present, opinions are different as to the maximum size of endocytosable nanosystems (probably, the upper bound is 500 nm);[21] also, it is unknown whether the size of the nanosystem is related to the mechanism of endocytosis.[235] However, it was reported that trapping of nanosystems by tumour cells is more efficient compared to normal cells, because the former to a greater extent require useful substances owing to higher metabolic activity and the growth rate.[236]

As mentioned above, biovectors and fragments of polymer molecules or functional groups attached to the surface of nanosystems can form chemical bonds with complementary molecules or receptors; this favours trapping of nanosystems by cells. A positive charge on the surface of nanosystems facilitates their penetration through the cell membrane because of the reaction of cationic groups of ligands with charged fragments on the membrane surface.[230] Among polymeric substances, polycations are more preferred for trapping.[237–239] However, it was found that particles with negatively charged surface covered by adsorbed low-molecular-mass compounds (DMSA, citric acid) can also be trapped readily. This was explained[163] by non-specific adsorption of negatively charged particles on the membrane surface and subsequent clustering because of the lack of steric repulsion.

Drugs are attached to the surface of nanosystems in such a manner that they release upon a change in the conditions (*e.g.*, pH, charge, *etc.*), which accompanies penetration of the nanosystem from the extracellular medium into the cell. The bond between the MNP and the drug should maintain stability to the interaction with biological fluids outside the cell and break down upon trapping. For instance, the bond between anticancer agent doxorubicin and the surface of a magnetite-based nanosystem broke in mild acid conditions (pH 5.3).[240] Nanosystems modified by a specific signal peptide can be incorporated into the cell nuclei.[241] To reach the cell nucleus containing genetic information, a drug should penetrate the nuclear membrane either in passive (through diffusion) or in active manner through a specific ionic channel called the nuclear pore (active transport). Detailed description of the mechanism of active trapping is available in the literature.[115,242]

Studies on penetration of various nanosystems (including those containing MNPs) through the cell membrane and on how to hold a MNP on the cell surface allow one to obtain structural and dynamic information on cells and cell membranes.[101] Effective trapping of MNPs is an important condition for their utilization in magnetic resonance imaging and for drug delivery.

3. Excretion of nanosystems from the body: the rate and routes

As a nanosystem is introduced into the human body, its surface contacts blood, other biological fluids and organs. The smaller the nanosystem and the more neutral and more hydrophilic its surface the higher the probability to avoid the action of the reticuloendothelial system and to increase the residence time of the carrier in the circulatory system. The reticuloendothelial system is part of the immune system and protects the organism from foreign materials, favours neutralization of adverse effects of some metabolytes, *etc.* Problems concerning protection of nanoparticles from the immune system and the possibilities of excretion of nanoparticles are intensively studied.[124,243] Cells that protect organisms by ingesting particles, bacteria as well as dying or dead cells, whose surface can be covered with specific receptors are called phagocytes. Trapping of nanosystems by phagocytes favours their excretion and disintegration. Trapping of a nanosystem by phagocytes as function of the electrostatic, steric repulsion and van der Waals forces acting between the particle and the phagocyte cell membrane was simulated.[244] Within the framework of the developed model it was shown that more hydrophobic particle is trapped faster than less hydrophobic one.

Nanosystems for drug and gene transportation and for magnetic resonance imaging should have low toxicity, be stable to the action of the reticuloendothelial system (to have time to accumulate in the target tissue) and have sufficiently long residence times in the circulatory system.

The half-life of nanosystems in blood is determined by their physicochemical properties, *viz.*, the particle size and shape and by the coating density and morphology.[245] Usually, hydrophobic particles are coated with hydrophilic polymers that act as steric barriers and can reduce the adsorption of opsonins (substances that signal phagocytes to ingest foreign materials; these are, *e.g.*, immunoglobulin G and components of the complement system) on the nanosystem.[184,246] The circulation time is often increased using biodegradable substances, *e.g.*, dextran and its derivatives,[92,247–249] polymers with flexible hydrophilic chains (in particular, PEG[250–252] having a low toxicity and immunogenic inferiority), poloxamers and poloxamines.[187] The dependence of the behaviour of PEG-coated iron oxide MNPs on the size of the magnetic core and on the polymer chain length was studied.[197] It was shown that the circulation time increases as the molecular mass of PEG increases and the nanoparticle size decreases.

The nanoparticle size influences the excretion rate and pathways of nanosystems from the body. Particles less than 10 nm in size can rapidly spread over the intracellular space and are excreted with urea.[155] As the nanoparticle size increases, no renal clearance takes place while the recognition by phagocytes occurs more readily; as a consequence, MNPs can accumulate in liver, spleen and lymph nodes.[253] Particles smaller than >200 nm are accumulated in liver and spleen; liver macrophages occupy the particles until their decomposition.[254] Nevertheless, transportation of

nanosystems to liver and spleen is of interest for magnetic resonance imaging of these organs.

Particles 10–100 nm in size are optimum for intravenous administration; they are small enough and can penetrate through thin capillaries in tissues, they have the longest circulation times and better reach the targets. The authors of a review [15] proposed that particles 5–10 nm in diameter are best candidates for biomedical studies. The efficiency of utilization of such particles is influenced by their concentration, surface characteristics and the properties of the medium.

It was shown that positively charged particles are faster removed from blood and reach liver and spleen, while negatively charged particles are characterized by long residence times and are mainly accumulated in the lymph nodes.[255] This is probably due to higher ability of cells to trap positively charged nanosystems. The surface of nanosystems introduced into the body can be involved in ionic interaction with charged objects in the medium. It also can interact with blood proteins, *e.g.*, fibrinogen. *In vitro* experiments [188] demonstrated efficient adsorption of fibrinogen on magnetite MNPs stabilized by phosphate–citrate buffer. Adsorption of protein on the surface of nanosystems can be reduced by preliminarily covering the surface with high-molecular-mass PEG chains. For instance, the amount of albumin adsorbed on the surface coated with PEG6000 was three times smaller than in the case of PEG750 coating.[174]

The shape of nanosystems determines their stability and distribution in the body. Unlike spherical particles, disk-shaped, cylindrical and hemispherical particles are less intensively trapped by phagocytes, can pass through capillaries and are adsorbed on the capillary walls. Compared to spherical particles, oblate spheroidal particles have longer residence times and are characterized by better penetrability, which can probably be explained by their self-organization in blood flow.[229, 256]

VI. Multifunctional coatings on magnetic nanoparticles

Design of biomedical nanosystems functionalized by biovectors and drugs is the most intensively developing area in bionanotechnologies. Nanosystems can have one magnetic core or a number of magnetic cores under a common coating based on biological or synthetic materials. The behaviour of nanosystems in the body is determined by the nature, stability, toxicity, and magnetic controllability of coatings (see Section V.1).

1. General requirements for nanoparticle coatings for biomedical applications

Successful utilization of MNPs in biology and medicine requires that the surface-coating materials be hemocompatible, thromboresistant, stable (no desorption from the surface of MNPs should occur in the body) and bear functional groups to attach drugs or biologically active substances. The properties of the coating influence the interaction of the nanosystem with biological media, the residence time in the circulatory system and trapping of the nanosystem by cells. General requirements for nanoparticle coatings are considered in more detail below.

a. Hemocompatibility and thromboresistance

Key factors determining the biocompatibility of a nanosystem include the hydrophilicity of its surface, as well as the charge and size of the nanosystem.[257] Particles with hydrophobic surface are prone to aggregation in water; therefore they are rapidly excreted by the reticuloendothelial system (see Section V.3). Hydrophobic nanosystems are characterized by *in vivo* lifetimes of the order of a few minutes (and even seconds), which makes them of limited use for drug delivery. Unlike hydrophilic particles, hydrophobic ones are faster trapped by macrophages and accumulated in particular organs. This allows hydrophobic nanosystems to be used to enhance image contrast in magnetic resonance studies of lymph nodes, liver, spleen or inflamed regions.[115, 131, 134]

Negatively charged coatings exhibit good biocompatibility; positively charged particles demonstrate better incorporation into cells.[239, 255] In the design of coatings, one should keep in mind that the surface charge of a nanosystem introduced into the body can vary owing to ionization of the surface groups and adsorption of charged biological molecules.[185]

The size of nanosystems determines the residence time in the circulatory system and the possibility of incorporation into cell structures.[21, 214, 234, 258] The size of nanosystems usually depends on the type of coating. In most cases, MNPs with inorganic coating are smaller than 100 nm in size (*cf.* more than 100 nm for polymer-coated MNPs).[235, 259] The biocompatibility of nanosystems can depend on the mass of the coating molecule.[174, 260]

Particular problems are solved by choosing coatings with optimum combination of the transport properties and biocompatibility.

b. Stability of coatings

Nanosystems introduced into the body should be stable and keep their coating continuous. Particular methods for anchoring coatings depend on their chemical nature. For instance, coatings can be anchored on the surface of MNPs through bonds between their functional groups and the surface and through covalent cross-linking of the coatings physisorbed on MNPs. Polymeric coatings can be stabilized through formation of cross-links,[261, 262] three-dimensional polymerization of monomers on the surface of nanoparticles [263] and formation of a polymeric layer due to chemical bonds formed between functional groups and the surface of nanoparticles.[264, 265]

c. Protection of the surface of MNPs

Coatings should isolate the surface of nanoparticles and thus protect them from the influence of external factors including oxidation which can affect the magnetic properties and prevent reactions of MNPs with components of biological media. Oxygen-impermeable coatings are thought to provide reliable protection of nanoparticles.

d. Stability of nanoparticle dispersions

Self-association of nanoparticles leading to their aggregation and agglomeration is governed by the thermodynamic reasons and magnetism. Coatings on MNPs should prevent self-association in nanoparticle dispersions using electrostatic forces or steric factors.

e. Functional groups

Coatings should improve targeting of nanosystems at organs, tissues and cells and at endocytosis. This is achieved using particular functional groups (constituents of coatings) or biovectors attached to the surface of nanosystems. Also, functional groups can be used to attach drugs to the surface of nanosystems.[266] If nanoparticles favour the formation of reactive oxygen species, the nanoparticle surface can be modified with antioxidants.[21]

e. Magnetic controllability of nanosystems

Coatings on MNPs make it possible to improve the magnetic controllability of nanosystems through clustering of particles (see Section IV) or fabrication of magnetic coatings (see below). The magnetic controllability of MNP clusters is higher than that of individual core/shell particles.

2. Fabrication of coatings based on various compounds

Coatings for MNPs are made of organic synthetic (polymers, surfactants) and natural (proteins, peptides, carbohydrates, *etc.*), inorganic (noble metals, carbon, oxides) and organoelement (organosilicon) compounds.

Components of coatings are chosen taking into account the presence of functional groups to be bound to the surface of MNPs, cross-linking of coatings and/or attachment of biovectors. Biovectors can specifically interact with targets (organs, cells, antigens cell surface, *etc.*). For instance, a PEG-containing polymeric coating was modified with biotin whose terminal group is responsible for the recognition of the nanosystem by neutravidin.[267] By analogy, the surface of nanosystems can bear fragments of avidin, neutravidin and streptavidin molecules. Avidin–biotin recognition can be realized involving biotin from the biological medium. Fabrication of coatings providing avidin–biotin recognition was documented.[12, 90, 268, 269] Yet another kind of biovector is transferrin which binds to receptors on the cell surface and thus switches on endocytosis.[270, 271]

The efficiency of nanosystems in vector delivery is determined by the drug loading capacity and by specific features of biovector release.

Biovectors and drugs can directly bind to coatings using cross-linking agents (glutaraldehyde, maleimide, carbodiimide) or the so-called 'click chemistry' approach.[54, 272] Practical implementation of the last-mentioned method can be exemplified by azide–alkyne binding: one group resides on the nanoparticle surface while the other one is a functional group of the molecule being attached.[273] In particular, glutaraldehyde was used for grafting trypsin to the surface of a chitosan-coated maghemite MNP.[274] A number of methods for chemical modification of the MNP surface bearing amino groups were developed. For instance, various heterofunctional binders and molecules with terminal functional groups were proposed.[275] This pattern allows anhydride, amine, hydroxyl, carboxyl, thiol and epoxy groups to be bound to the surface of MNPs. Grafting of molecules of low-molecular-mass compounds to the surface of MNPs led to nanosystems specific to endothelial cells, liver tumour cells (this was demonstrated *in vivo*) and activated macrophages. A review[276] presents a long list of bifunctional cross-linking agents, which can be used to attach drugs containing one (doxorubicin, epirubicin, daunorubicin, idarubicin, pirarubicin) or more amino groups (mitomycin, bleomycin, peplomycin) to the surface MNPs modified with amino groups.

Coatings based on organosilicon compounds whose molecules contain NH_2, OH, CN and SH groups have a great potential for modification and attachment of biovectors. Commercially available Dynabeads particles ('Dynal', Norway)[277] can exhibit either hydrophobic or hydrophilic properties owing to the presence of different functional groups. Surface modification by epoxy and tosyl groups is aimed to obtain hydrophobic particles, which enables subsequent smooth adsorption and binding of ligands with retention of the functional activity of enzymes and the native conformation of unstable proteins after anchoring. Hydrophilic particles are obtained by functionalization of the surface by amino and carboxyl groups. Hydrophobic Dynabeads particles are optimum for binding antibodies in the affinity isolation of proteins or organelles.

a. Synthetic polymers and surfactants as coatings

Synthetic polymeric or surfactant-based coatings can be formed on the available MNPs and during the synthesis of MNPs. Such coatings can provide electrostatic (through variation of the surface charge of MNPs) and/or steric stability of MNP dispersions in neutral media. Polymers and surfactants can be either chemically bound to or physisorbed on the surface of MNPs. The coating should be thick enough to prevent agglomeration of particles under the action of magnetic forces. When fabricating coatings for biomedical applications, it is desired to utilize molecules that can not only stabilize colloidal nanosystems in dispersion, but also bear functional groups for subsequent anchoring of coatings on the surface of MNPs as well as grafting of biovectors and drugs. Usually, low-molecular-mass are inappropriate to this end. Steric stabilization of MNPs for biological applications is achieved using hydrophilic polymers.

Hydroxyl, carboxyl, phosphate, phosphonate, sulfate, sulfonate or other groups, as constituents of surfactant or polymer molecules, favour bonding to MNPs bearing hydroxyl groups on the surface.[7, 156, 157, 159, 164, 278] Stabilization and modification of MNPs using compounds containing these groups can be achieved in both water and organic media depending on the chemical structure of compounds. For instance, poly-(*N*-isopropylacrylamide),[269] polyacrylic and polymethacrylic acids[279, 280] and citric acid were used to fabricate hydrophilic coatings on cobalt and magnetite MNPs.[162] Citric acid imparts hydrophilic properties to the particle at the expense of carboxyl groups not bonded to the surface of MNPs. Free carboxyl groups can be used to attach drugs, *e.g.*, doxorubicin.[266] Fabrication of coatings using modification of natural and synthetic compounds (*e.g.*, PEG, glucose, saccharose, starch) by functional groups for binding to the surface of MNPs is widely used. Compounds based on PEG and orthophosphoric acid efficiently bind to the surface of metal oxide MNPs.[264] Dopamine and ascorbic acid molecules forming coordination bonds with the surface of iron oxide MNPs can be attached to terminal groups of various compounds. A dopamine-modified biphosphonate molecule was linked to the surface of magnetite MNPs.[160] Also, iron oxide MNPs were modified by poly(ethylene glycol) using the bond between dopamine and the MNP surface.[265]

Additives whose molecules contain carboxyl (citric, gluconic, disulfanylsuccinic, oleic, lauric acids), phosphate (phosphorylcholine, dihexadecyl phosphate, *etc.*), phosphonate (dodecylphosphonic acid) or sulfonate groups (hexa-

decylsulfonic acid) introduced during the synthesis of nanoparticles strongly influence their growth rate, magnetic properties and form a shell on the surface.[14, 92, 281–283]

Synthetic polymeric and surfactant coatings are used to transfer MNPs synthesized in organic media to aqueous dispersions. Transfer of MNPs from a hydrophobic to a hydrophilic medium is carried out using the so-called phase-transfer agents, *i.e.*, compounds containing hydrophilic (*e.g.*, hydroxyl) groups.[184] Phase-transfer agents based on PEG and 1,2-bis(dichlorophosphino)ethane were proposed.[284] Poly(ethylene glycol) makes maghemite MNPs hydrophilic. Magnetic nanoparticles coated with hydrophobic hydrocarbons can be stabilized in aqueous dispersions by amphiphilic compounds that bind to the hydrophobic shell of MNPs.[285]

Coating of MNPs with surfactant molecules may lead to formation of bilayers and micelles hundreds nanometres in diameter. Magnetic nanoparticles can be incorporated into liposomes and a liposome can include more than one particle.[286] These nanosystems are biocompatible; their shape and structure favour trapping by cells and the constituent MNPs ensure good magnetic controllability. A drug incorporated into liposomes can be released on heating. Magnetoliposomes releasing doxorubicin on heating to 42 °C under the action of alternating magnetic field were reported.[287]

There are three general methods for polymer-coating of MNPs. One of them involves preliminary modification of a polymer (or utilization of the properties of functional group in the polymer molecule) for binding to the surface of nanoparticles. Corresponding examples were considered above.

The second method involves polymerization of monomers on the surface, *viz.*, the surface of nanoparticles is covered with an initiator, a monomer is added to the dispersion, and relevant polymerization conditions are established.[288] In particular, MNPs were coated using polymerization of styrene and acrylic acid.[263] Magnetite MNPs were stabilized by oleic acid and oleylamine and then treated with ozone which led to the formation of peroxy and hydroperoxy groups (initiators of radical polymerization) on the surface of nanoparticles. It was proposed to coat dispersions of citrate-stabilized magnetite MNPs with silica, functionalize the surface with C=C groups using 3-(trimethoxysilyl)propyl methacrylate and then carry out polymerization of *N*-isopropylacrylamide in the presence of *N*,*N*′-methylenebis(acrylamide) (MBAA).[289] The nanoparticle size after the polymerization of *N*-isopropylacrylamide depended on the amount of the cross-linking agent. This method was used for fabrication of a high-density coating anchored on the surface of MNPs. A poly(methyl methacrylate) coating on magnetite MNPs was prepared using a phosphonic acid based surface initiator.[169]

The third method is to form cross-links. We will consider methods of preparation of various synthetic polymeric coatings on MNPs, in particular, methods of cross-linking of polymeric coatings.

Hydrophilic coatings on MNPs are often fabricated using reactions in microemulsions including microemulsion polymerization.[290] Coatings obtained by this method have the same thickness at all points.

Note that polymeric coatings can form on the surface of individual MNPs or combine several MNPs to form large clusters.[171, 289]

To date, the list of polymers used to fabricate coatings on MNPs includes PVP, polyacrylic acid, polyacryl cyanoacrylate, polyaniline, polypyrrole, poly(methylidene malonate), polyglycolic acid, polymethacrylic acid, polyethyleneoxide, polylactic acid, poly-ε-caprolactone, polystyrene, ethylcellulose and their copolymers, *e.g.*, poly(ethylene oxide)-*block*-poly(methacrylic acid), poly(ethylene oxide)-*block*-poly(propylene oxide) (so-called poloxamers), poloxamines, *etc.*[14, 17, 18, 291] Each coating for biomedical applications has its own advantages and drawbacks. Examples of utilizing a number of these polymers for fabrication of coatings were considered above. Now we will dwell on three synthetic polymers widely used for coating MNPs for biomedical applications, *viz.*, PEG, polyethyleneimine (PEI) and polyvinyl alcohol (PVA).

Coatings made of poly(ethylene glycol), which is a hydrophilic, water soluble, biocompatible polymer, remain stable for a long time even at high ionic strengths of solutions and in a wide range of pH values. A number of studies on the possibility of using PEG to improve the biocompatibility of iron oxide MNPs and the circulation time in blood are available.[180, 292, 293]

The terminal group of PEG chain can be modified to ensure the chemical functionalization of coatings or ionic stabilization of particles in dispersions.[290, 294]

PEG-coated nanosystems are characterized by long (a few hours) residence time in the blood circulation system.[92, 186, 250–252] The circulation time of nanosystems in the body increases if the polymer forms a 'glomerule' on the surface of MNPs because coatings with close-packed polymer chains and high molecular mass reduce the adsorption of blood plasma proteins.[174] PEG-coated nanosystems are difficult to recognize by the reticuloendothelial system; therefore, they are inapplicable to magnetic resonance imaging of macrophages. PEG coatings can be formed not only on the surface of MNPs, but also on the phospholipid micellar shell of iron oxide MNPs.[286, 295] Their functional groups can be used for binding proteins, oligonucleotides and peptides. Systems for methotrexate delivery to glioma cells are based on PEG-coated MNPs.[296]

It should be noted that the bond between PEG and the surface of MNPs is noncovalent;[12] therefore, attempts of many researchers are aimed at anchoring PEG coatings on the nanoparticle surface. A cross-linked coating on the surface of MNPs was prepared by modification of methoxy-PEG5000 with maleic anhydride to give an ester bond.[290] The coating was formed in reverse micelles in the presence of MBAA; the nanoparticle size was 40–50 nm. Another way of binding PEG to iron oxide MNPs involves modification of the polymer with functional groups providing stronger bonds with the surface, *e.g.*, dopamine.[265] A method of coating the carbonized surface of MNPs with stable biocompatible PEG coatings was proposed.[297] The carbon surface was preliminarily modified with carboxyaryldiazonium tosylate and subsequent formation of *para*-substituted benzoic acid which forms a covalent bond with PEG.

Polyethyleneimine coatings contain a large number of amino groups and form cationic complexes which readily react with negatively charged surfaces and substances.[176] This polymer is positively charged; hence, it interacts with proteins and exhibits cytotoxicity; however, these polymers can be used for DNA and RNA delivery to cells.[115, 298] The release of nucleic acids in the case of magnetofection using

PEI was reported.[299] PEI-coated iron oxide nanoparticles appeared to be efficient for *in vivo* magnetofection.[300] Recent advances in the use of PEI for magnetically guided gene delivery were analyzed in a review.[115] However, fabrication of PEI-based coatings on iron oxide MNPs is a complicated task because adsorption of the polymer is accompanied by the formation of large clusters in a wide range of pH values.[178, 301]

Maghemite MNPs were stabilized in aqueous and physiological solutions with alternating PEI/[poly(ethylene oxide)-*co*-polyglutamine acid] coatings and nanoparticles less than 50 nm in size with charges of different magnitude and sign were obtained.[302] Experiments with rats demonstrated the applicability of these nanoparticles for magnetic resonance imaging.

Polyethyleneimine was covalently bound to magnetite MNPs whose surface was preliminarily modified with citric acid.[190] The reaction between PEI amino groups and citric acid carboxyl groups was carried out in the presence of 3-(3-dimethylaminopropyl)-1-ethylcarbodiimide hydrochloride and *N*-hydroxysulfosuccinimide.

An analysis of published data on the use of PEI-coated MNPs showed that the PEI coatings are promising for biomedical applications, but they should be additionally modified because of the large surface charge.

Polyvinyl alcohol coatings on the surface of MNPs are hydrophilic and can be prepared by different methods including the formation during the synthesis of nanoparticles. In particular, the addition of PVA during co-deposition of iron salts in a basic medium gave PVA-coated magnetite MNPs with a narrow size distribution.[167] Using glutaraldehyde, one can obtain a polymer gel based on PVA.[303] As the gel is dried, the surface of MNPs is coated with a biocompatible film. Functionalization of PVA can be aimed at anchoring drugs to the surface of nanoparticles.[239] PVA-coated iron oxide MNPs can be used to improve the reliability of diagnosis of neurodegenerative diseases.[304]

A microemulsion method for fabrication of thermoresponsive nanosystems for drug delivery with no cross-linking agents was proposed.[305] Nanosystems are comprised of a core (a number of magnetite MNPs and a hydrophobic drug) and a biocompatible shell (PVA and Pluronic F127 poloxamer) 5–8 nm thick. The coating remains stable owing to the formation of a system of hydrogen bonds and can release drugs on heating to 40–50 °C.

b. Natural polymeric coatings

Natural compounds to the greatest extent meet the biocompatibility requirements; therefore, magnetic nanosystems with coating based on natural polymers seem to be most promising for biomedical applications. The diversity of chemical structures and functional groups enables various applications of natural macromolecular compounds, in particular, vector drug delivery to biological targets, hyperthermia and magnetic resonance imaging. The most abundant macromolecular compounds include dextran and its derivatives, starch, chitosan, glycosaminoglycans, proteins and peptides. Also, phospholipids forming a micelle-like shell on MNPs were used.[286]

Consider main research avenues in the design of coatings on MNPs using natural macromolecular compounds.

Coatings made of carbohydrates and their derivatives have long been used for fabrication of biocompatible nanosystems for image contrast enhancement in magnetic resonance imaging.[28, 131] Carbohydrates include dextran and its derivatives, starch, lactose, glucose, mannose, *etc*. Dextran is a biocompatible polysaccharide polymer comprising solely α-D-glucopyranose units; the chain length and degree of branching can vary.[17, 247] Dextran-coated nanoparticles have low toxicity and are characterized by long residence times in the circulatory system.[140, 247] A dextran coating on magnetite MNPs can be formed during the synthesis of these nanoparticles using, *e.g.*, epichlorohydrin as cross-linking agent.[261] A method for fabrication of dextran coatings on iron oxide MNPs under the cation of microwave field was reported.[306] Coatings can be formed during the synthesis of nanoparticles and in the subsequent steps.

Dextran derivatives, *e.g.*, carbomethylated dextran and carboxydextran, are used as coatings on MNPs.[248, 249] Chemical modification of dextran with ammonium salts makes it possible to obtain amino-functionalized dextran in order to provide further bonding to biovectors and drugs, *e.g.*, anthracyclines (daunomycin and doxorubicin).[307] Biovector molecules (peptides, antibodies, proteins, oligonucleotides) can be grafted to dextran-coated MNPs.[308]

Chitosan is a basic, non-toxic, hydrophilic, biocompatible and biodegradable polymer.[12, 14] Chitosan coatings on magnetite MNPs can be prepared during the synthesis of nanoparticles (in the microemulsion process)[191] or in the subsequent steps. Similarly to amino-functionalized dextran, chitosan is used for grafting biovectors.[309] Trypsin was grafted on chitosan-coated maghemite MNPs using glutaraldehyde.[274] Nanosystems coated with chitosan as well as microbeads based on chitosan and iron oxide MNPs can be used in magnetic resonance imaging and the development of drug nanocarriers.

The use of starch for coating MNPs during their synthesis makes it possible to obtain clusters 200–300 nm in size. Subsequently, they can be modified to form carbon coatings.[181]

Protein and peptide coatings favour reduction of cytotoxicity and improvement trapping of nanosystems by cells,[247] react with various compounds, exhibit enzymatic activity, can participate in antibody–antigen and receptor–substrate interactions and their functional groups can be used for anchoring biovectors and drugs. For instance, the surface of magnetite nanoparticles stabilized with sodium oleate was modified with insulin in the presence of 3-(3-dimethylaminopropyl)-1-ethylcarbodiimide as cross-linking agent.[310] Specific proteins anchored on the surface of nanosystems improve transfer through biological barriers; *e.g.*, apolipoprotein favours transfer of MNPs through the blood–brain barrier.[311] When using proteins as coatings, usually, it is necessary to retain their activity to the greatest possible extent.[312] Thin coatings on MNPs can not be formed owing to the large size of protein molecules. To eliminate this problem and retain a small hydrodynamic radius of nanoparticles for *in vivo* applications, an active fragment of a protein is grafted to the surface of MNPs. Peptides on the surface of MNPs can perform some functions inherent in proteins. Peptide sequences may include groups that favour transmembrane transport of MNPs and groups responsible for intracell targeting and the possibility of anchoring drugs.[90, 313] Consider the main methods of fabrication of protein and peptide coatings on MNPs.

Traditionally, this is achieved by applying an intermediate coating while proteins and peptides are attached as biologically active substances. The surface of magnetite

MNPs was modified with aminosilanes with subsequent anchoring of protein molecules using glutaraldehyde.[292] The same cross-linking agent and chitosan as intermediate coating were also used.[274]

Yet another method of binding MNPs to proteins is to add proteins during the synthesis of MNPs. For instance, a biofunctional trypsin coating on $Fe_3O_4-\gamma$-Fe_2O_3 particles was prepared.[314] Trypsin was introduced during the synthesis of MNPs in aqueous solution. However, the stability of this coating is doubtful because the protein macromolecules are not covalently bound to the surface of MNPs.

Chemical adsorption or physisorption of proteins on the surface of MNPs are widely used coating techniques. In particular, immunoglobulin G was covalently bound to the surface of magnetite MNPs using 3-(3-dimethylaminopropyl)-1-ethylcarbodiimide hydrochloride.[315] Nanosystems coated with IgG selectively interacted with pathogen cell walls and acted as probes in diagnosis. The same carbodiimide derivative was used to anchor trypsin to the surface of magnetite MNPs.[316]

Attempts to fabricate protein coatings by adsorption of proteins on MNPs followed by covalent bonding of the proteins using bifunctional cross-linking agents were reported. In particular, BSA was adsorbed on the surface of MNPs in the presence of carbodiimide; however, protein molecules were desorbed from the surface of nanoparticles.[262] It should be noted that immobilization of protein molecules on the surface of MNPs using bifunctional cross-linking agents can be accompanied by undesired formation of a polydisperse ensemble of particles. This is due to the fact that cross-linking is a non-selective process which involves not only molecules adsorbed on individual nanoparticles, but also molecules in solution as well as molecules belonging to different MNPs.

The authors of this review proposed a method for preparation of protein coatings on the surface of magnetite MNPs based on physisorption of proteins and immobilization of the adsorption layer by the free-radical mechanism.[317] This utilizes the ability of proteins to enter free-radical reactions with the formation of intermolecular covalent bonds.[318] Free radicals appear upon adding an initiator (*e.g.*, hydrogen peroxide) to the solution containing MNPs and protein. Since free radicals are produced only in the region of contact between the surface containing variable-valence metal ions and the initiator, covalent cross-linking that improves the strength of the coating is localized exactly within the layer consisting of the protein adsorbed on the surface. In turn, the protein coating thus formed precludes spread of radicals in solution. This excludes the possibility of formation of (i) cross-links between protein macromolecules in solution and (ii) a polydisperse ensemble of particles due to cross-linking of the protein molecules immobilized on different MNPs.

A method for preparation of biocompatible coatings on MNPs based on microwave-assisted solid-phase polymerization of monomers (amino acids) was proposed.[319] Heating of powdered MNPs and solid monomer was accompanied by polymerization of amino acids on the surface.

c. Organosilicon coatings

There is a number of methods for coating nanoparticles with silicon compounds. This makes it possible to form stable thin shells on MNPs with retention of magnetic properties. Silica coatings on iron,[320] nickel,[321] cobalt,[322, 323] $CoFe_2O_4$,[324] $MnFe_2O_4$,[325] iron oxide[289, 326–328] *etc.* MNPs were reported. Most often, they are prepared by the sol–gel method (Stöber method),[329] namely, coatings on MNPs are fabricated as a result of hydrolysis and condensation of intermediates $Si(OR)_{4-x}OH_x$. The role of precursors is often played by tetraalkoxysilanes, *e.g.*, $Si(OEt)_4$.[289, 328, 330] A silica coating can be formed on gelatin-sheathed MNPs with gelatin NH_2 and COOH groups being used for chemical bonding.[331] In the case of iron oxide MNPs, silica can be bound to the surface through hydroxyl groups (Fig. 5). The coating thickness depends on some factors including the concentration of ammonium salt acting as catalyst, the precursor : water and ethanol : water ratios in the reaction mixture and the concentration of iron oxide MNPs.[174, 320, 332] The particle size in coatings varies from 5 to 200 nm depending on the ammonium salt concentration and the precursor : water ratio.[26] Individual silica-coated MNPs are obtained at pH 7.5.[54] It should be noted that in some cases the formation of organosilicon coatings on MNPs is accompanied by clustering.[174] It was proposed to prepare coatings on cobalt MNPs using block copolymers containing polydimethylsiloxane and polymethylvinylsiloxane units and a precursor of the formation of the surface layer with trimethoxysilane groups.[333] The block copolymers were added during the synthesis of MNPs. The process results in the formation of nanoparticles with a narrow size distribution.

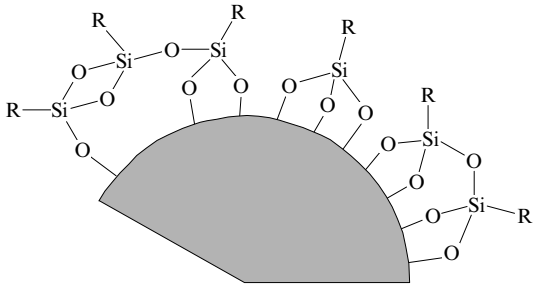


Figure 5. Formation of an organosilicon coating on the surface of a MNP containing hydroxyl groups.

Utilization of substituted propyltrialkoxysilanes, *e.g.*, 3-(aminopropyl)triethoxysilane[180, 334–336] and 3-(propylsulfanyl)triethoxysilane,[330] makes it possible to obtain coatings bearing NH_2 and SH groups, respectively. Functional groups on coatings are used for further modification of nanosystems with synthetic and biological molecules. The terminal group of the coating compound determines the stability of systems in aqueous dispersions. For instance, silanes with amino, hydroxyl and carboxyl terminal groups, as well as silanes modified with PEG can be used to prepare dispersions for biomedical applications.[337, 338] Coatings bearing amino groups can be modified with glutaraldehyde to bind to proteins.[292] Coating of magnetite MNPs in the presence of 3-(bromopropyl)trichlorosilane made it possible to carry out polymerization of polyaniline on the surface of the coating.[339] Note that silanes can also be added upon the formation of the primary silica coating. Using 3-(trimethoxysilyl)propyl methacrylate, polymerization of *N*-isopro-

pylacrylamide on the surface of silica-coated magnetite MNPs in the presence of MBAA was conducted.[289, 340]

Silica coatings are also obtained using silicic acid.[334, 341, 342] The size of nanosystems thus formed can be as large as a few hundreds of nanometres at particular $SiO_2 : Fe_3O_4$ ratio or as a result of repeated coating procedure. In order to introduce drugs (doxorubicin and ibuprofen) into the nanosystems, mesopores 2–4 nm in size were created in the coating using trimethylcetylammonium bromide.[54, 180]

Coated particles with narrow size distributions are also prepared by the emulsion method. Reactions are carried out within micelles or reverse micelles.[343–346] Here, a difficulty consists in the need for separating coated MNPs from large amounts of surfactants. The emulsion method was used to prepare a 2-nm thick coating on MNPs.[346] Methods of fabrication of porous coatings on magnetite MNPs with an average pore size of 1.5 nm and introduction of drugs (gentamicin) into the pores were reported.[347] Coating of $CoFe_2O_4$ and $MnFe_2O_4$ MNPs with silica in reverse micelles led to a decrease in the magnetization of nanoparticles.[325]

Pyrolysis of tetraethoxysilane with an iron salt in methanol gave hollow spherical silica-coated maghemite particles 150 nm in diameter.[348] More recently, the same authors reported the preparation of silica beads with high content of maghemite.[349] A versatile method for the formation of silica coatings on nanoparticles of different compositions was proposed.[350] It involves modification of the nanoparticle surface with PVP.[350]

Negative charges of silica-coated nanoparticles favour their dispersion in water under the action of Coulomb repulsion forces (no surfactants). Silica shells can be formed on both hydrophilic and hydrophobic MNPs.[337, 350] The thickness of the silica coating can be varied; however, uniform coatings are difficult to obtain. Yet another drawback of silica coatings consists in their porosity and thus the possibility of diffusion of various compounds towards the surface of MNPs. The advantages of silica coatings include stability, hydrophilicity and the possibility of functionalization. Functional groups on the surface of a coating determine the possibility of its modification and utilization for anchoring biovectors. Nanosystems based on MNPs coated with organosilicon compounds can be used for gene separation,[351] as contrast agents in magnetic resonance imaging and for controlled drug (in particular, methotrexate) delivery.[352, 353]

d. Inorganic coatings

Inorganic coatings are based on carbon, noble metals, magnetic materials and metal oxides.[354–358] They protect MNPs from the action of the environment, form thin dense layers and can be prepared readily in contrast to complex methods of organic synthesis. Below we will consider only a few approaches to the use of inorganic substances as coatings for biomedical applications.

Carbon coatings. Carbon coatings are more chemically stable than polymeric or organosilicon ones. Carbon layers protect the surface of MNPs from the action of chemical factors including oxidation.[18, 359] Carbon coatings are formed by various methods, *e.g.*, arc discharge, pyrolysis, chemical vapour phase deposition, *etc.* Fabrication of carbon coatings on iron, cobalt and nickel MNPs using electric arc between carbon electrodes was reported.[360]

In some cases, carbon coatings on MNPs have different thickness at different points and clustering occurs. A carbon coating on magnetite MNPs with a starch shell was formed by pyrolysis at 200 °C.[181]

Magnetically controlled iron–carbon particles containing 44%–90% of iron were obtained by plasma-chemical method at 6000 °C.[361] The average particle size was about 1000 nm and the saturation magnetization varied from 43 to 82 emu g^{-1}. The systems were of low toxicity. Owing to the developed surface (specific surface area of the order of 60–146 $m^2\ g^{-1}$) particles exhibited high adsorption capacity. Iron carbon particles can be used for adsorption of antibiotics, toxins, pathogens and for drug (*e.g.*, doxorubicin) delivery.

A new hydrothermal method for preparation of carbon coatings followed by annealing at 1000 °C was proposed.[362] Owing to the chemical stability of the coating and high magnetization (235 emu g^{-1}) FeCo/C particles obtained by this method are more promising for biomedical applications than FePt, Fe_2O_3 and Fe_3O_4. However, biocompatibility of the FeCo/C nanoparticles is achieved only upon subsequent surface modification with dextran (the particle diameter is about 150 nm). A procedure for PEG application onto carbon-coated iron MNPs was proposed.[297]

Noble metal coatings. Metal-coated magnetic particles (Metal = gold, silver, platinum, palladium, cobalt, copper) are used in biomedical studies and catalysis. Particles coated with noble metals (platinum, gold) are stable in neutral and acid aqueous media as well as stabile to oxidation.[363–365] Gold coatings are most interesting for biomedical applications[366] Recently, this metal has been used in therapy; therefore, gold-coated MNPs combine therapeutic effect and magnetic controllability. Gold is coated on the surface of iron,[364, 365, 367] cobalt,[368, 369] iron oxide,[370–373] *etc.* MNPs. Gold-coated cobalt particles with specified size and shape were prepared.[368] Multifunctional hybrid nanoparticles comprised of gold and magnetite particles are thought to be new efficient agents for magnetic resonance imaging.[374] The structure of the hybrid nanoparticles allows one to separately modify the surface of each type of nanoparticles. The gold/iron hybrid particles were described in Ref. 375.

Metal coatings can be prepared by different methods including laser irradiation,[376] chemical reduction of corresponding metal compounds on the surface of MNPs or on the surface of MNPs with organic or organosilicon coating[26, 373, 377] and by reductive transmetalation (replacement of metal atom).[363, 367] The thickness of the coating depends on the coating procedure. For instance, platinum-coated cobalt particles less than 10 nm in diameter with narrow size distribution were prepared.[363] A gold coating on magnetite MNPs in a 3-(aminopropyl)triethoxysilane shell was reported.[373] Particles were characterized by high saturation magnetization ($\sim$63 emu g^{-1}). The average particle size was 30 nm. Based on nanoparticles modified with amino groups, anisotropic particles coated with noble metals (silver,[378] gold[379]) were fabricated. To this end, the growth of gold seeds adsorbed on the surface nanoparticles was completed in a solution containing a reducing agent and the noble metal compounds. Noble metal coatings can be fabricated on the surface of MNPs in microemulsion systems and reverse micelles.[370, 380]

Although coatings are stable, their thickness may be insufficient to preclude agglomeration of MNPs caused by

magnetic interactions. Nevertheless, the gold surface can be modified with chemical and biological agents to ensure stability of aqueous MNP dispersions in such coatings.[373] Gold coatings are suitable for attachment of thiol groups. For instance, PEI2000 was grafted to the gold surface modified with thiol groups and a cross-linking agent.[381] The surface of Fe_3O_4/Au hybrid particles was modified with HS-PEG5000.[382] Using cross-linking agents, functional groups can be grafted to the gold-coated surface for subsequent modification and attachment of biovectors.[370, 383]

Magnetic coatings. From the aforesaid it follows that iron oxides are more readily biofunctionalized than other magnetic materials. This makes it possible to use them as coatings to improve the biocompatibility and bonding to drugs and biovectors. By varying the thickness of the coating one can change the magnetic properties (see Section II.1). In particular, iron oxides are used as coatings for FePt[384, 385] and cobalt[159] nanoparticles; ferrite $CoFe_2O_4$ is a coating for magnetite MNPs.[386] Magnetic coatings can also be prepared on nonmagnetic particles to make them magnetically controlled. For instance, cobalt coatings on platinum MNPs[387] and magnetite coatings on gold MNPs[374] were reported. The structures of binary systems comprising magnetic and nonmagnetic components can differ from the core/shell structure. For instance, particles containing cobalt and platinum representing a 'nano-alloy' with the chemical composition $CoPt_3$ were prepared.[363]

3. Brief conclusions

Summing up, coatings on MNPs for biomedical applications can be prepared using various compounds including organic, inorganic and organosilicon ones. Coatings based on organosilicon compounds are widely used owing to a large number of well-known methods of chemical modification of their surface. The possibility for using MNPs coated with organosilicon compounds in various biomedical applications is due to the diversity of functional groups on the surface. However, silica coatings are porous; this may favour undesired adsorption activity of these nanosystems in biological media and incomplete isolation of the surface of MNPs. Organic coatings have a great potential for modification. It was shown that PEG, PVA, PVP, dextran and its derivatives, chitosan and starch improve the biocompatibility of nanosystems and increase their residence time in the body. Among inorganic coatings, gold and carbon can be highlighted. These coatings can be prepared on the surface of MNPs during their synthesis or formed on the available particles using various physical and chemical methods.

The properties of the compound chosen to be the coating determined the amount of MNPs in the nanosystem and, therefore, its magnetic controllability. An important requirement for the coating design methods is to prepare nanosystems with narrow size distributions. This can be done using microemulsion processes. The properties of the coatings determine the toxicity of nanosystems and their interaction with biological media.

VII. Toxicity

Nanosystems may exhibit neuro-, cardio-, genetic and hepathotoxicity; they can provoke inflammatory response and an oxidative process leading to cell necrosis.[243] In some cases, nanoparticles can provoke allergic reactions.[388] Nanosystems can damage cell membranes and organelles and spread potentially toxic components in the body. An important step in the design of systems based on MNPs is to carry out a benefits/risks assessment. At present, there are numerous methods for studying biocompatibility; however, rigorous toxicity assessment of MNPs is still impossible. Problems concerned with the applicability of the traditional toxicity tests of nanomaterials and the transferability of toxicologic data from animals to humans remain topical.[389]

Toxic effects of nanosystems depend on their chemical compositions, size, shape and on the properties of coatings. *In vivo* experiments showed that iron oxide MNPs are relatively safe, are not accumulated in vital organs, rapidly excrete from the body and are less toxic than other MNPs.[390, 391] This is probably due to the possibility for iron oxide MNPs to decompose to iron ions obeying the regularities of cell homeostasis.[155, 390, 392–394] The Fe^{3+} cation is nontoxic; in contrast to this, the Fe^{2+} cation is toxic because it can participate in reactions resulting in free radicals or reactive oxygen and nitrogen species.[395, 396] It was proposed[397] to use mixed ferrites MFe_2O_4 (M = Co, Mn, Ni, Zn) instead of magnetite for hyperthermia; the former exhibit suitable magnetic properties, are physically and chemically stable and exhibit low toxicity. Maghemite is less toxic than magnetite because it contains no Fe^{2+} ions.[155] Excess iron in the body predetermines a number of diseases including neurodegenerative ones.[398]

Toxic cations can be released when using metal and ferrite MNPs.[399] To reduce toxic effects, the surfaces of such particles are modified with antioxidants.[21] The response of respiratory system in mice to metal nanoparticle exposure (MNPs represented beads 30 nm in diameter with iron core and 2–3 nm thick magnetite shell) was studied.[400] Inhalation of nanoparticles provoked an inflammatory process, oxidative stress and hypoxia. It was proposed to inhale MNPs into lungs in combination with some biologically active substances to reduce inflammatory activity of MNPs.

Modification of the surface of MNPs by coating nontoxic organic (chitosan, dextran, liposomes) and inorganic (carbon, gold) coatings is the main method of minimizing toxic effects.[15, 26, 129, 193, 401] The properties of coatings (thickness and stability, charge, nature of functional groups and hydrophobicity) determine the residence time of particles in the body and the excretion pathways, as well as the ability of particles to be trapped by cells and provoke abnormal changes in them. For instance, it was stated[402] that a magnetic fluid based on magnetite stabilized with oleic acid is characterized by low toxicity and can be used for biomedical purposes; however, long-term toxic effects should be taken into account. The maximum tolerance dose and the absolute lethal dose of this magnetic fluid determined in the experiments with rats are 150 and 450 mg kg^{-1}, respectively. An analysis of functional groups on the surface of MNPs in the body was carried out.[24] High concentration of cations on the surface of the nanosystem may lead to loss of the continuity of the cell membranes and violation of the function of the organelles.[238] Cationic particles have higher cytotoxicity and more probably provoke hemolysis and aggregation of thrombocytes compared to electrically neutral and anionic particles.[21, 403] There is a

number of reviews on the cytotoxicity of nanosystems.[399, 404]

Usually, nanosystems decompose upon trapping by phagocytes. Hardly degradable materials are harmful. The shape of particles influences their trapping by phagocytes. It was shown that aspherical particles are trapped, pass through capillaries and are adsorbed on the capillary walls less readily than oblong particles.[229, 256] It was established that oblong particles (carbon nanotubes) behave like asbestos fibres and provoke frustrated phagocytosis.[405]

The reactivity of particles towards biological medium depends on their specific surface area. Therefore, if a nanosystem contains toxic substances, smaller nanosystems introduced into the body are much more hazardous than large ones.

At present, toxicology of MNPs and nanosystems based on them are still poorly studied; however, the number of studies in this field continuously increases.

VIII. Conclusion

Design of nanosystems with multifunctional coatings for biomedical applications is a rapidly progressing area in modern nanotechnology. Nevertheless, despite the fact that the idea of binding drugs to the surface of MNPs was suggested more than three decades ago and there is considerable research activity in the field of vector drug delivery using MNPs, this investigation line is far from being practically implemented as yet. The idea to deliver nucleic acids to damaged cells using MNPs was proposed much more recently but there is also still a long way to clinical application. There is also little *in vivo* data in the field of MNPs application for hyperthermia. To date, the greatest progress is made in utilizing MNPs as contrast agents for magnetic resonance imaging.

We believe that slow advancement of these promising areas in bionanotechnology is due to various reasons. First, it is difficult to determine 'optimum parameters' of MNPs for biomedical applications. Next, there are some problems concerning the synthesis of MNPs with preset properties. In spite of progress made during the last 10 – 15 years, progress in the development of methods for synthesis of MNPs with different size and chemical composition, the synthesis of MNPs with narrow size distribution and high degree of crystallinity still remains difficult because the seeding and nanoparticle growth mechanisms scratch the surface. It should be noted that MNPs are often synthesized in organic solvents. In this connection, organic media should be replaced by water and particles should be concentrated.

The development of stable dispersions of MNPs is also a challenge. Particles are prone to agglomeration and tend to decrease the free energy due to the large surface : volume ratio. There are a number of compounds used to form stabilizing coatings on MNPs, but their properties in biological media are still poorly studied. Often, coatings on MNPs should protect nanosystems from the action of the reticuloendothelial system in order to deliver them to specific tissues. The chemical compositions of such coatings are hard to predict, but it is desired that the coating process and subsequent immobilization of coatings on MNPs be standardized to allow one to prepare stable nanoparticle dispersions or powders readily soluble in water.

The number of methods of investigation of nanosystems based on MNPs is limited. Therefore, the behaviour of such objects in biological media is difficult to predict.

A particular, complicated step in the development of nanosystems for biomedical applications is to fabricate stable biocompatible coatings and to anchor biovectors and drugs on their surface. Polymeric coatings should be immobilized on MNPs through chemical bonding and be stable in biological media. The biovector and drug anchoring techniques should become more reproducible and provide the possibility of introducing controlled amounts of these substances. Considerable attention should be paid to physicochemical drug release mechanisms in the body.

At present, the chemical composition of the protein coating formed upon introduction of a nanosystem into the body, the efficiency of cell trapping, side effects of nanosystems on the function of organism are difficult to predict. Problems concerned with the toxicity of nanosystems are also poorly studied.

We expect that the synthesis of MNPs followed by coverage with multifunctional modifier coatings will attract increasing attention of researchers working in physics, chemistry, biology and medicine. In the nearest decades, MNPs may become a unique, highly efficient noninvasive tool in various biomedical applications including therapy of malignant tumours. Localization of nanosystems in particular regions of living organisms owing to magnetic controllability and the presence of biovectors on their surface will make it possible to carry out diagnosis using magnetic resonance imaging and perform targeted drug and gene delivery in the early stages of disease.

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