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Nanocomposites of medicobiologic destination: reality and perspectives for oncology.

Review

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Analysis is given of the ways of creation of polyfunctional medicobiologic nanocomposites with multilevel hierarchic architecture that would be applicable for recognition of microbiological objects in biologic media, targeted delivery and deposition of drugs in target organs, diagnostics and therapy of diseases on the cellular level. The physicochemical experimental data, as well as medicobiological studies, confirm their perspective for preparation of new medicinal forms with complex (cytotoxic, immunotherapeutic and hyperthermic) activity.

Проанализированы пути создания полифункциональных медико-биологических нанокомпозитов с многоуровневой иерарархической архитектурой, предназначенных для распознавания микробиологических объектов в биологических средах, направленного транспорта и депонирования лекарственных препаратов в органах-мишенях, диагностики и терапии заболеваний на клеточном уровне. Физико-химические экспериментальные данные, медико-биологические исследования подтверждают их перспективность для изготовления новых лекарственных форм онкологических препаратов комплексного (цитотоксического, иммунотерапевтического и гипертермического) действия.

1. Introduction

There is no doubt that nowadays malignant tumours represent one of the most difficult medicobiological problems. Positive results of therapy of cancer patients depend on early diagnostics and timely medicinal cure or surgical operation. However, in most cases not only the transformation of a normal cell into malignant form, but also the formation of tumor nidus remains unnoticed until the clinical observation of disease [1]. Therefore the actual objective of researchers all over the world is the search for new methods of early diagnostics of tumours and the creation of medicines with selective effects upon malignant cells. Great hopes for achievement of these goals are laid upon the development of nanotechnologies [1–3].

At present nanotechnologies are the most dynamical project in the field of science and medicine [4]. In oncology their application can allow diagnostics, prevention and treatment of cancer patients before the clinical observation of disease [1, 3, 5]. For many centuries humanity has been looking for remedies to avoid the tumour diseases. However, the outstanding successes of fundamental sciences in the field of cancer biology, chemistry, and pharmacology achieved in the last decades did not result in the desired successes in clinical oncology. This primarily refers to the selectivity of accumulation and, respectively, to the speci-

ficity of known cytostatic agents. Undoubtedly, it is striking that only a tiny fraction (1:10000–1:100000) of the intraperitoneally introduced dose, even on the base of such modern carriers as monoclonal antibodies, reaches the specified target [3, 6]. In principle, nanotechnologies allow delivering the medicine directly to a small clone of malignant cells [3, 7]. This also applies to the contrast agents for visualisation [8, 9].

The problem of creation of new antitumour remedies is one of the most actual in modern oncology. The absence of selectivity of known medicines, as well as their high general toxicity, stimulate both further search for new compounds and improvement of the existing medicine forms with the purpose of increasing their selectivity and safety [3]. The modern nanotechnologies can not only solve this task, but also to realize multivector character of the antitumor action mechanism of nanocomposites [10]. It has been long understood that targeted delivery of the active agents is a fundamental part in the development of medicines. A wide range of systems for drug delivery should improve their stability and therapeutical concentration in a target tissue, as well as directed absorption and effects influence on a molecular target [11]. In most cases such nanocomposites include compounds known as agents for pharmaceutical correction or direct cytostatic action [2, 5, 11]. In certain cases the compositional reconstruction of known substances involving one or another type of nanoparticles can improve their targeted accumulation upon peroral introduction [12]. The inclusion of nanoparticles with given properties can provide both protection of the mucus cover of gastrointestinal tract and protection of the substances themselves from degradation in locations with severe pH conditions. The surface modification of nanoparticles allows covalent or noncovalent attachment of a number of different compounds [5, 11]. As successfully used active nanosystems for targeted action one can note folates [13, 14], as well as monoclonal antibodies for the surface proteins of cancer cells and/or integrines [2, 11, 15]. Using such active nanosystems, a composite can enter the reaction with molecules on the cell surface and also on the level of intercellular cascades of signal transmission, participating in the regulation of broken signal pathways [11]. A number of nanosized systems for medicine transport have already reached the stage of the first clinical trials [16, 17].

Considering the whole complex of affecting factors in the process of pharmacokinetics directed by a nanocomposite, there are good reasons to hope that a broad range of antitumour action mechanisms can be involved in the achievement of effect: from antisignal ones to hyperthermia [3, 10, 11]. With the use of multistep nanocomposites and their electromagnetic maintenance, the real possibility is emerging to modulate pharmacokinetic processes in tumour tissue in the system *in vivo*. The most significant constituent in this process is the possibility of additional creation of uniform and controlled hyperthermic effect using external magnetic field [18, 19]. The synergism of anticancer effect is reached with the account of cytostatic action of temperature and also owing to additional and accelerated release of the anticancer agent from a nanocomposite. Using a ferromagnetic material composed of iron and nickel alloy and vanadium dioxide, one can create a nanocomposite (dielectric-conductor, conductor-dielectric) that can maintain the temperature at Kurie point (43–45°C) in automatic regime basing on the phase transition.

Ideally, nanocomposites can have longer cycling time not losing their efficiency. Accounting for their selectivity, new medicinal systems [19–30] can significantly increase not only the efficiency of anticancer drug [23–30], but also largely improve the quality of life of patient [1–5].

In this work the scientific and applied aspects have been analyzed related to the modern approaches to creation of new medicine forms through chemical design of polyfunctional nanocomposites of medicobiological destination with multilevel architecture and a programmed complex of functions.

2. Synthesis and properties of magnitosensitive polyfunctional nanocomposites

The scheme of chemical design of polyfunctional nanocomposites. The initial material for the chemical design of nanocomposites was one-domain magnetite (Fe_3O_4) with crystallite size of 7–60 nm. It is known that magnetite is biogenic and is removed satisfactorily from the organism. In the hierarchic structure of nanocomposite it works as a magnetosensitive carrier of medicinal remedies and transformer of the energy of high-frequency external magnetic field into thermal energy by creation of hyperthermal zones, it has reactive surface that allows carrying out the chemical design

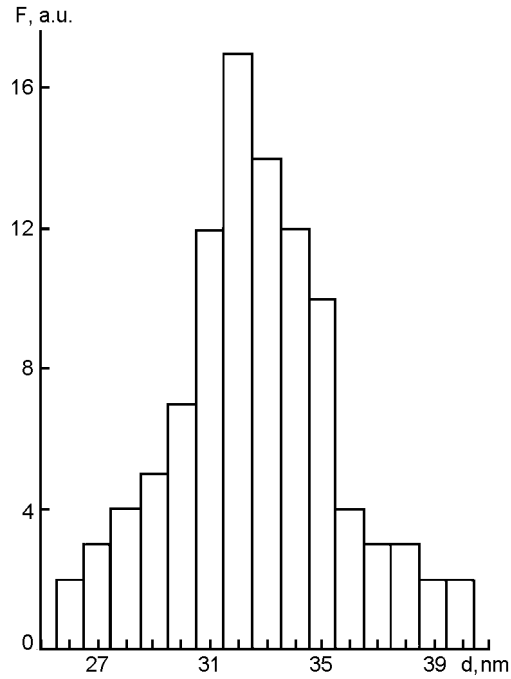


Fig. 1. Size distribution of magnetite particles (F is given in arbitrary units, d is particle diameter, nm).

and construction of the necessary nanoarchitecture.

Magnetite was stabilized by the modification of the surface with a biocompatible cover. The presence of such cover increases the specific surface of the carrier and allows the necessary chemical functionalization.

The next stage in the creation of nanocomposites is related to the immobilization of chemical, immunotherapeutical and diagnostic preparations, as well as neutron capture agents. The capsulation of nanocomposites was carried out by dextran, gelatin, poly vinyl alcohol (PVA), polyvinyl pyrrolidone (PVP).

The nanocomposites synthesized according to the scheme described may be used for magnetodirected delivery of medicinal preparations, diagnostics, chemical and immunotherapy, as well as hyperthermia, particularly, on the cellular level.

The basic technological stages of creation of polyfunctional nanocomposites were elaborated according to this scheme [23–30]. Synthesis nanotechnologies promising for practical application were developed, physical and chemical properties of magnetosensitive nanocomposites and magnetic fluids on their base were studied — such as colloidal systems that are stable in water, phosphate buffer and physiological solution, as well as biofunctionalizated magnetic nanocomposites and liquids for pre-clinical studies (Table 1).

Let us consider in more detail the basic stages of synthesis and functionalization of experimental samples of polyfunctional

Table 1. List of synthesized and studied experimental samples

Magnetosensitive polyfunctional nanocomposites	Magnetic liquids	Biofunctionalized magnetosensitive nanocomposites	Biofunctionalized magnetic liquids
Fe_3O_4/PAA/CP	Fe_3O_4/ol.Na	Fe_3O_4/PAA/Ig	Fe_3O_4/DMSA/SMCC/Iiq
Fe_3O_4/γ-APS/CP	Fe_3O_4/ol.Na/CP	Fe_3O_4/γ-APS/Ig	
Fe_3O_4/HA/CP	Fe_3O_4/ol.Na/PEG	Fe_3O_4/HA/Ig	
Fe_3O_4/HA-Ag/CP	Fe_3O_4/ol.Na/PEG/CP	Fe_3O_4/HA-Ag/Ig	
Fe_3O_4/Gd_2O_3	Fe_3O_4/DMSA	Fe_3O_4/γ-APS/CD-95	
$Gd_xFe_{3-x}O_4$	Fe_3O_4/Gd_2O_3/ol.Na	Fe_3O_4/γ-APS/CP/CD-95	
	Fe_3O_4/Gd_2O_3/ol.Na/PEG		
	Fe_3O_4/DMSA/CB	Fe_3O_4/PAA/CD-95	
		Fe_3O_4/PAA/CP/CD-95	
		Fe_3O_4/HA/CD-95	
		Fe_3O_4/HA/CP/CD-95	

Abbreviations in Table 1: PAA — polyacrylamide, γ-APS — γ-aminopropylsiloxane, DMSA — *meso*-2,3-dimercaptosuccinic acid, SMCC — sulfosuccineimidyl-4-(N-maleimidomethyl)-cyclohexane-1-carboxylate, HA — hydroxyapathite, CB — carborane, ol.Na — sodium oleate, CP — cisplatine, PEG — polyethyleneglycol, Ig — immunoglobuline.

nanocomposites, as well as results of their studies.

The synthesis and properties of magnetite. The synthesis of magnetite was carried out by the coprecipitation of iron salts according to the reaction:

$$Fe^{+2} + 2Fe^{+3} + 8NH_4OH \rightarrow$$

$$\rightarrow Fe_3O_4 + 4H_2O + 8NH_4^+. \quad (1)$$

In order to obtain the nanoproduct yield not less that 85 % (mass) of monodomain particles Fe_3O_4 with the assigned size distribution, we used the cryochemical technique of heterogenic synthesis of magnetite on the phase boundary: one solid — frozen solution of iron(II) sulfate and iron(III) chloride salts, and the second liquid — the ammonia solution of fixed concentration. As distinct from homogenic synthesis, it is possible to change the growth conditions of nanoparticles in the synthesis process. Samples of nanocrystallic magnetite were obtained, with specific surface area changing in dependence on the parameters of initial solution within ~ 40–180 m^2g^{-1}. The average size of the particles was dependent on the synthesis conditions, with values of 6–50 nm; the interval of size distribution can be technologically controlled, being sufficiently narrow (Fig. 1).

The advantage of the technique proposed consists in the possibility of obtaining nanomaterials with high content of monodomain particles with the assigned size distribution, and in simplification of the ultradisperse product separation process. Nanocrystalline one-domain magnetite (fraction of 20–50 nm) was obtained also by the method of solid phase synthesis in the inert atmosphere. As initial compounds, we used metal-organic compounds of bi- and trivalent iron. Magnetite obtained by the solid phase method showed the highest values of coercitive force (150–240 Oe), with the specific surface of ~40 m^2g^{-1}.

The investigation of hyperthermal effect. In order to clarify the possibilities for creation of hyperthermal zones, we investigated the nonresonance (thermal) influence of electromagnetic radiation (λ = 3 cm) on the model animal muscular tissues *in vitro* injected by nanodisperse magnetite (Fig. 2). The mass of the tissue samples was 5 g. Samples 2 and 3 were differed by the content of small quantity of grease layer. The area of radiation was ~2 cm^2.

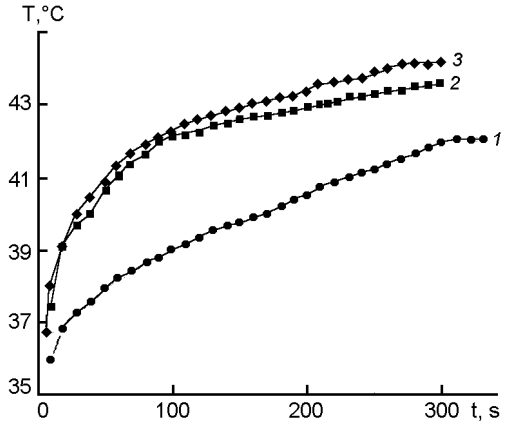


Fig. 2. Temperature of muscular tissue samples as function of treatment time: *1* — initial, *2*, *3* — with magnetite.

Table 2. Calculated values of magnetic field gradient for carrier retention

Required magnetic field gradients for blood vessels (diameter $d \leq 3$ mm)		
Vessel type	Blood flow velocity, U	Gradient level
Veins	0.1 cm/s	$\nabla H \geq 7$ kOe/cm
Arteries	0.4 cm/s	$\nabla H \geq 28$ kOe/cm

It was discovered that in the case of injection of Fe_3O_4 nanoparticles into muscular tissues (0.1 mass %) the absorption of electromagnetic radiation increased, and the rate of their heating increased by 1.5–2 times (in comparison with the control samples without magnetite) and reached 4 grad/min at the irradiation power value of 0.5 Wt.

Analysis of transport and retention conditions of magnetosensitive carriers of medicinal preparations with the help of magnetic field. Theoretical estimations were made of conditions of transport and fixation of magnetosensitive nanocomposites (the carriers of medicinal preparations) with the aid of external magnetic field. It was shown that retaining nanocontainers with drugs could be reached even in the large magistral blood vessels when magnetic systems were optimally chosen. The realized calculations and the obtained gradient levels show the actual possibility of delivering and retaining magnetic carriers in target organs (Table 2).

At the same time, in the view of the complexity of the problem of the controlled transport of medicinal preparations one can draw a conclusion that solving specific therapeutical problems with the help of magnetic carriers demands realization of many-

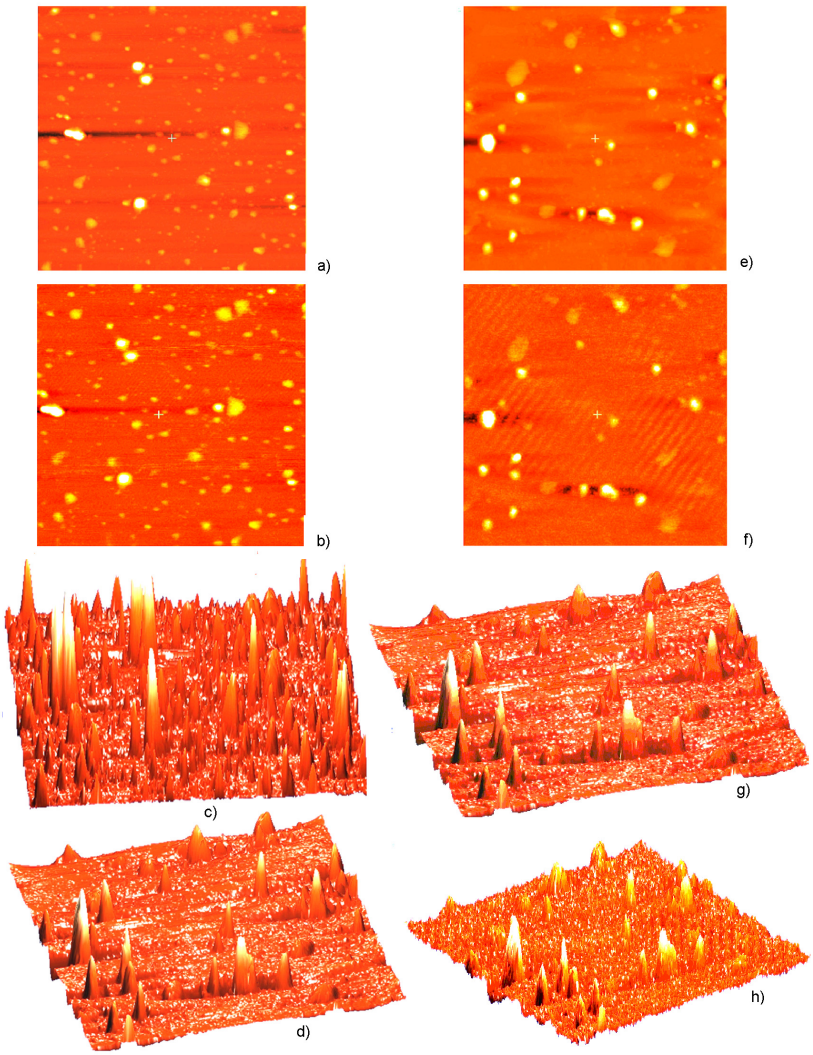


Fig. 3. Atomic force (a, c, e, g) and magnetic force (b, d, f, h) images of nanocomposite arrays Fe_3O_4/PAA (a — d) and Fe_3O_4/γ-APS (e—z) in formats 2d (a, b, e, f) and 3d (c, d, g, h).

sided theoretical investigations on the model systems, as well as carrying out experiments involving cellular cultures and animals.

Creation of a biocompatible covering on the surface of nanosized magnetite. In order to stabilize the properties of magnetosensitive carriers and to increase their specific surface area, they were modified with biocompatible coverings.

The nanotechnology of magnetite surface modification with polyacrylamide consists in the formation of covering on the surface of nanosized magnetite by polymerization of acrylamide with sewing agent N, N′-methylene-bis-acrylamide in high-frequency glow discharge plasma. It was shown from the analysis of kinetic curves of titration of double carbon (C=C) bonds that the full polymerization of acrylamide layer was realized in 2 min.

The samples of Fe_3O_4/PAA nanocomposites were investigated by the atomic force and magnetic force magnetic nanoscopy. Fig. 3 (a–d) shows the atomic force (a, c) and magnetic force (b, d) images of Fe_3O_4/PAA nanocomposite arrays (a–d) in the formats 2d (a, b) and 3d (c, d).

The dried powder-like samples with a broad size distribution of the particles and their aggregates (from 8 nm to 1 μm) were used for the investigations. The images of nanocomposites obtained by the atomic force and magnetic force methods are apparently in good accordance, showing coordination of the magnetic and "force" phases, as well as small thickness and sufficiently uniform

of cross-linked polyacrylamide around the magnetite particles.

The modification of the magnetite surface by γ-aminopropylsiloxane was carried out by the liquid phase method in toluene. As a result of polycondensation reaction the surface of magnetite got base properties because of the grafting of γ-aminopropyl groupes at their concentration of 24 μmol/m^2 when the specific surface area was 90 m^2g^{-1}. The atomic force (e, g) and magnetic force (f, h) images of Fe_3O_4/γ-APS nanocomposite arrays (e–h) in the formats 2d (e, f) and 3d (g, h) are shown in Fig. 3. As in the previous case, one can draw a conclusion about the coordination of magnetic and "force" phases and small thickness and sufficiently uniform distribution of γ-aminopropylsiloxane layer around the magnetite particles.

The modification of the magnetite surface with hydroxylapatite was carried out to give high biocompatibility to Fe_3O_4 magnetosensitive carriers. It was determined that hydroxylapatite phase on the surface of carrier was characterized by the correlation Ca/P = 1.67, which corresponds to the stoichiometry of the reaction of its creation.

The average size of magnetite and hydroxylapatite crystallites was calculated in accordance to (311) and (002) X-ray diffraction peaks, respectively, with the use of Sherrer formula. The thickness of hydroxylapatite layer on the surface of magnetite nanoparticles was ~4 nm, which was evaluated by the area ratio of Fe2p- and Fe3p-lines (studied by X-ray photoelectronic spectroscopy) and the increase in the Fe_3O_4/HA nanocomposite mass (~30 %).

The modification of the magnetite surface with *meso*-2,3-dimercaptosuccinic acid is one of the variants to solve the problems of colloidal system stability in aqueous medium, the material biocompatibility, the immobilization of necessary compounds through thiol and carboxyl functional surface groups.

Intermolecular disulfide bonds of DMSA bound with the surface increase the stability of the cover. The aqueous sol obtained from the magnetite nanoparticles covered with DMSA is stable in the broad range of pH values (3–11) in aqueous and phosphate buffered systems. It was found that the concentration of SH groups was 0.019 mmol/m^2 when the specific surface area was 130 m^2g^{-1}.

The results of measuring the distribution of the particles of Fe_3O_4/DMSA/water magnetic liquids by the method of dynamic light scattering on zeta potential and the average size of nanocomposite particles are shown in Table 3.

Table 3. Zeta-potential and average particle size for magnetic liquids Fe_3O_4/DMSA/water

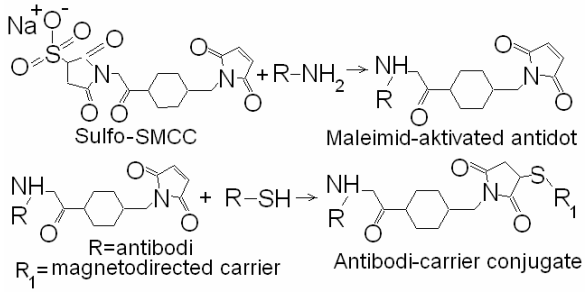
Zeta-potential, mV	–40.3
Zeta-potential distribution width, mV	9.84
Sample conductivity, mS/cm	0.129
Average size of particles, nm	40.5

The negative potential of the particles in magnetic fluids prevents their aggregation. With increasing pH, the zeta-potential diminishes because of the increase in the ionization degree of COOH and SH functional groups.

Biofunctionalization of nanocomposites. Biofunctionalization is an important stage in the creation of medico-biological nanocomposites, because it provides them with functions of recognition of microbiological objects in biological media and targeted delivery of medical preparations to them.

The immobilization of maleimide activated immunoglobulines on the surface of Fe_3O_4/DMSA was carried out to obtain the immunomagnetic nanosorbents and nanocomposites with recognition functions. We have developed a technique of magnetic liquid creation with simultaneous modification of the surface of magnetite particles with DMSA and immobilization of antibodies with the help of spacer molecules of sulfo-succinimidyl-4-(N-maleimidomethyl)- cyclohexan-1-carboxylate (sulfo-SMCC). It was shown that the chemical immobilization of antibodies on the surface of Fe_3O_4/DMSA nanocomposites may be realized by the interaction of the reactive sulfo-groups of the carrier with the maleimide functional groups of immunoglobuline (Ig) activated previously with sulfo-SMCC.

The two stage reaction of the immobilization of immunoglobuline on the surface of Fe_3O_4/DMSA nanocomposites by means of sulfo-SMCC spacer leads to the formation of specific complexes with antibody:



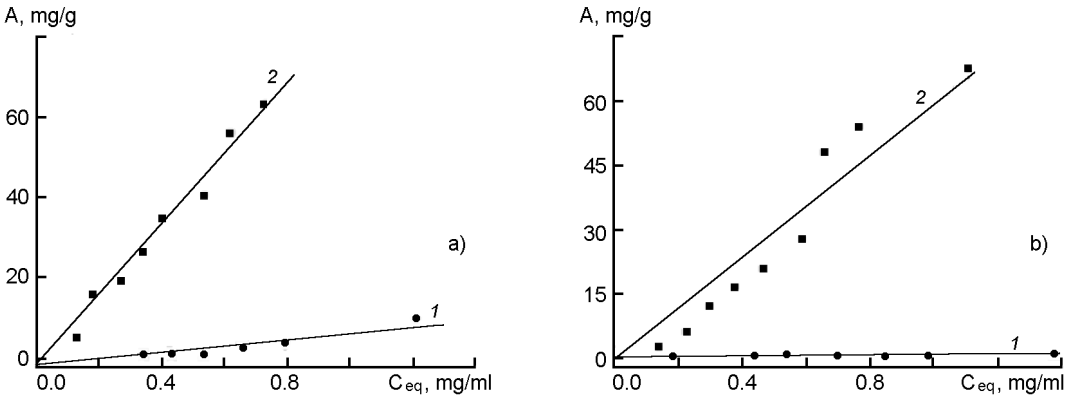
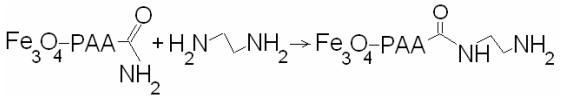


Fig. 4. Isotherms of nonspecific adsorption of human Ig (*1*) and covalent binding of the oxidized Ig (*2*) onto the surface of nanocomposites Fe_3O_4/PAA (a) and Fe_3O_4/γ-APS (b).

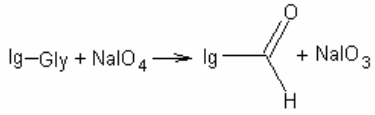
The isoterms of adsorption of maleimide activated immunoglobulines on the surface of Fe_3O_4/DMSA were investigated. The adsorption capacity value $A = 92.12$ mg/g, with the specific surface area $S_{specific} = 130$ m^2g^{-1}. The release of immunoglobuline, immobilized by means of sulfo-SMCC spacer, in the model medium was 8–12 % for 24 h.

The covalent immobilization of normal immunoglobuline of humans on the surface of Fe_3O_4/PAA and Fe_3O_4/γ-APS is based on the idea of binding immunoglobuline with the surface of nanocomposites by means of using the interaction of aldehyde groups (created on Ig molecules as a result of periodate oxidation of side carbohydrate chains) with aminogroups of the modificator of the carrier surface. This leads to formation of Schiff bases (imines).

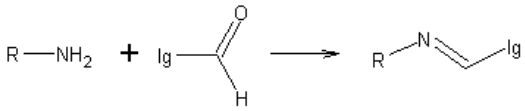
In the case of nanocomposites Fe_3O_4/PAA, covalent binding of Ig was carried out after activation of polyacrylamide surface with ethylenediamine to form reactive $-NH_2$ groups:



The oxidation of human normal Ig was carried out in acetate buffer-based solution of $NaIO_4$ according to the reaction:



The covalent binding of oxidized Ig with the surface of Fe_3O_4/PAA nanocomposites (activated with ethylenediamine) and Fe_3O_4/γ-APS was carried out in carbonate buffer based on physiologic solution according to the reaction:



The comparative studies of isotherms (Fig. 4, 5) of nonspecific (physical) adsorption of human normal Ig (Fig. 4, a, b, curves 1, Fig. 5) and covalent binding of the oxidized Ig (Fig. 4, curves *2*) onto the surface of nanocomposites were carried out. We calculated the coefficients of distribution (E) of immunoglobuline between the surface of Fe_3O_4/PAA, Fe_3O_4/γ-APS, Fe_3O_4/HA, Fe_3O_4/HA//Ag nanocomposites and the solution, as well as quantities of Ig immobilized at maximal concentration of its initial solution $C = 1.4$ mg/g (Table 4).

In the case of covalent binding of Ig the coefficients of distribution of Fe_3O_4/PAA and Fe_3O_4/γ-APS nanocomposites are higher than the respective coefficients for nonspecific adsorption by more than an order and reflect the equilibrium shift towards surface immobilization of substance.

It was shown by experiment that covalent binding of Ig according to the reaction proposed has essential advantages in comparison with nonspecific adsorption: the oriented fastening of antibody molecules and restriction of Ig desorption by hydrolysis velocity of Schiff bases.

The adsorption of Ig on the surface of Fe_3O_4/HA/Ag exceeds that on the surface of Fe_3O_4/HA in two investigated buffer systems. It may testify for nanoparticles of silver on the surface of composite to be additional adsorption centers. Considering high adsorption of immunoglobuline by Fe_3O_4/HA and Fe_3O_4/HA/Ag nanocomposites ($A = 34$–38 mg/g) in comparison with

Table 4. Quantity of human Ig immobilized on nanocomposite surface

Nanocomposite	$A_{phys.}$, mg/g	$E_{phys.}$, ml/g	E_{cov}, mg/g	E_{cov}, ml/g
Fe_3O_4/PAA	9.48	6.1	116.00	83.53
Fe_3O_4/γ-APS	1.18	0.92	67.41	59.51
Fe_3O_4/GA (phys.sol.)	34.0	47.2		
Fe_3O_4/GA (phosph.buf.)	25	111.36		
Fe_3O_4/GA/Ag (phys.sol.)	38.0	59.4		
Fe_3O_4/GA/Ag (phosph.buf.)	25.5	186.67		

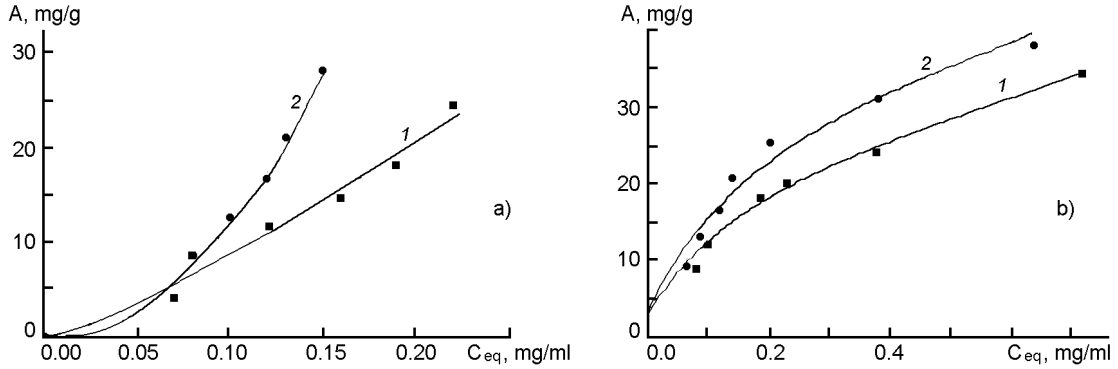


Fig. 5. Adsorption isotherms of human Ig on the surface of nanocomposites Fe_3O_4/HA and Fe_3O_4/HA/Ag from phosphate buffer (a) and physiological solution (b).

adsorption of nanocomposites functionalized by aminogroups (A = 1.18–9.48 mg/g), one can suppose formation of compact oriented packing of adsorbed antibodies on the surfaces of nanocomposites modified with HA and silver.

The immobilization of antibodies on the surface of nanocomposites magnetite-polyacrylamide, magnetite-γ-aminopropil-siloxane and magnetite-hydroxylapatite was carried out by the techniques described above.

We studied nonspecific (physical) adsorption and covalent binding of CD-95 monoclonal antibodies on the surface of Fe_3O_4/HA and Fe_3O_4/PAA, Fe_3O_4/γ-APS nanocomposites, respectively. As sensor molecules we used the monoclonal mouse antibody CD-95 against the human Fas-antigen of the isotype Ig1, kappa, DX2 clone produced by DakoCytomation (Denmark). The quantity of antibodies immobilized was determined by calibrated graph and the adsorption value A was calculated to be 165 μg/g for Fe_3O_4/PAA nanocomposites, 137.7 μg/g — for Fe_3O_4/γ-APS and 590 μg/g — for Fe_3O_4/HA.

One may remark that a magnetosensitive sensor carrier, when recognizing a specific microbiological object in a biological medium, for example a cell, is in direct proximity from it, or penetrates through a membrane in intracellular space. Then there is a possibility to determine its space location and to observe its time change by magnetic resonance methods. Using computer tomography, one can visualize this process and obtain three-dimensional images. The latter is used in practice, being especially important for early diagnostics of diseases and determination of exact sizes of tumours.

Immobilization of cysplatin at the surface of the nanocomposites. Cysplatin (CP) is an antitumour preparation on the base of platinum. The mechanism of its antitumour action is connected with bifunctional alkylating of DNA chains, which suppresses biosynthesis of nucleic acids and induces cell apoptosis.

It was shown that cytotoxic activity of cysplatin in the samples studied was preserved for one month.

Adsorption kinetics of CP at surfaces of the nanocomposites was investigated (Fig. 6).

It was shown that the adsorbed amounts of cisplatin (calculated for Pt^{2+}) at the surfaces of the nanocomposites Fe_3O_4/PAA, Fe_3O_4/γ-APS and Fe_3O_4/HA were 128 mg/g, 98.3 mg/g and 60.1 mg/g, re-

Table 5. Results of nanocomposite cytotoxicity studies

References	Number of dead cells, %									
	Effect	Fe_3O_4/ γ-APS+CP	Fe_3O_4/ γ-APS+ +CD-95	Fe_3O_4/γ-APS+CP+ +CD-95	Fe_3O_4/ PAA+CP	Fe_3O_4/ PAA+ +CD-95	Fe_3O_4/ PAA+CP+ +CD-95	Fe_3O_4/ HA+CP	Fe_3O_4/ HA+CD-95	Fe_3O_4/ HA+CP+ +CD-95
Cysplatine (CP)	25	31			38			48		
Antibody CD-95	10		20			21			27	
CP+CD-95	40			46			57			57

spectively. The major part of the drug is adsorbed during the first 2–3 h.

Release of the preparations from the nanocomposite surface

Kinetics of desorption of Ig immobilized on the surface of nanocomposites was studied with the use of physiological solution as a model medium. It has been shown that the release of immunoglobuline immobilized on the surface of Fe_3O_4/PAA and Fe_3O_4/γ-APS nanocomposites by covalent bond is going slowly and to lesser extent than in the case of nonspecific immobilization. The antibodies immobilized on the surface of Fe_3O_4/HA and Fe_3O_4/HA/Ag nanocomposites showed weak desorption in a model medium.

By investigating kinetics of release of cysplatine from the surface of Fe_3O_4/PAA nanocomposites in the physiological solution it was found that about 50 % of cytotoxic preparation was washed out in 80 min.

Investigation of cytotoxic effect of the magnetosensitive nanocomposites. We studied cytotoxic influence of the magnetosensitive nanocomposites with the adsorbed cytostatic preparation conjugated with monoclonal antibody (nanorobot models) upon viability of the cell line of human breast cancer MCF-7 (Table 5). For comparison, the influence of control samples on cell viability with the separately immobilized monoclonal antibodies and cytostatic drug was investigated. As a reference, we used: the pure nutritious medium; cysplatine in a concentration C = 2.5 μg/ml, corresponding the fourth dose of biological equivalent efficacy IC_{25}; monoclonal antibody CD-95 in C = 0.2 μg/ml (the dose used in curing is 10–30 μg/ml).

The biocompatibility of initial magnetite and Fe_3O_4/PAA, Fe_3O_4/γ-APS and Fe_3O_4/HA nanocomposites (modified carriers) to the said cell line was shown experimentally. The use of magnetic nanocomposites that comprise antitumour drug and monoclonal antibody CD-95 was accompanied by significant synergic effect of cytotoxic action. Their efficacy exceeds the combined action of the respective control doses of cysplatine preparations and antibody. So, it was shown that the efficacy of Fe_3O_4/PAA/CP/CD-95 and Fe_3O_4/HA/CP/CD-95 model nanorobots that comprise antitumour drug CP and monoclonal antibody CD-95 exceeds by 1.5–2 times the combined cy-

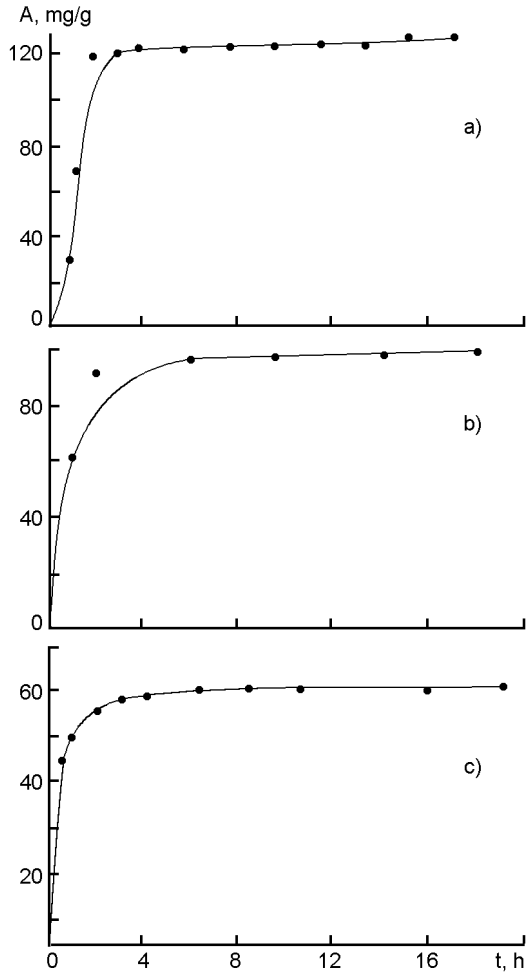


Fig. 6. Adsorption kinetics of cysplatine on the surface of nanocomposites Fe_3O_4/PAA (a), Fe_3O_4/γ-APS (b), Fe_3O_4/HA (c).

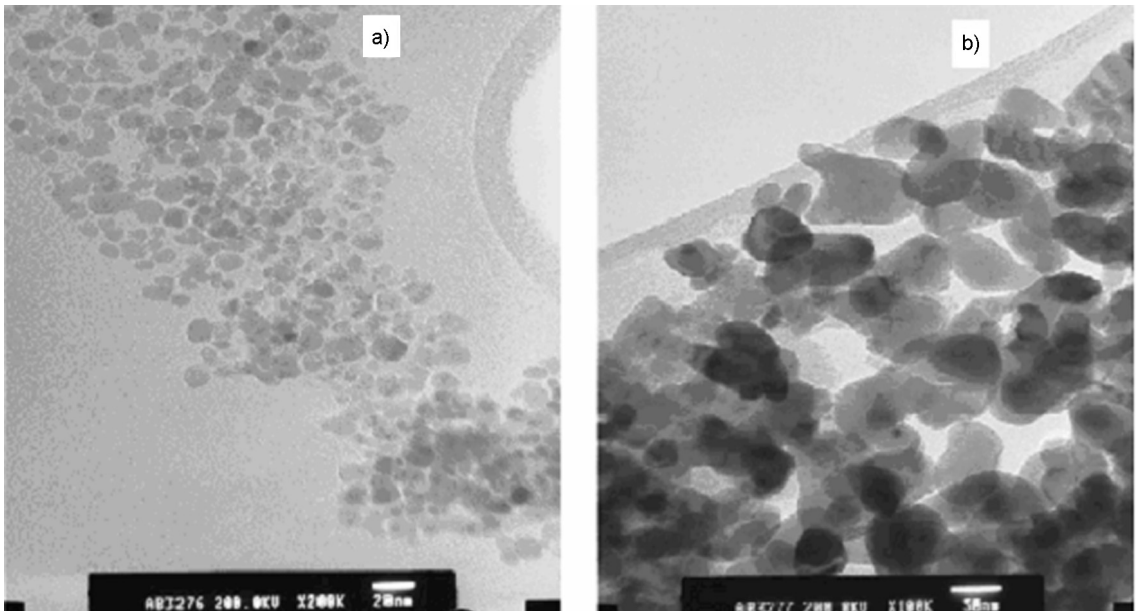


Fig. 7. Scanning electron microscopy images of dried magnetic liquids on the basis of Fe_3O_4. Scales: a — 20 nm, b — 50 nm.

totoxic action of the respective control doses.

The synergic effect of action of biologically active components of nanocomposites may be explained as follows.

Firstly, the targeted delivery of the complex "cytostatic drug — oriented monoclonal antibody" is carried out by the magnetosensitive carrier to tumour cells due to recognition of respective receptors on their surface. The cytotoxic effect of cysplatine is realized through formation of covalent bonds of preparation molecules with DNA. With this, cell death is caused by traumatic action of nanosized carrier onto cellular membrane which essentially enhances penetration of medicine preparation through the membrane barrier. Secondly, the optimized system ligand-receptor plays an important role in apoptosis of malignant cells. Binding with its receptor, the antibody launches the immunotherapeutical mechanism that leads to apoptosis.

So, the use of magnetosensitive polyfunctional nanocomposites that include antitumour drug and monoclonal antibody CD-95 allows one to realize recognition of specific cells and to reach cytotoxic effect with lower concentrations of medicine remedies, diminishing toxico-allergical influence of chemicotherapeutical preparation onto the organism as a whole.

Thus it was shown that the synthesized nanocomposites can fulfill such functions as magnetodirected delivery of drugs, recognition of microbiological objects in biological media, diagnostics, local chemical and immunotherapy, as well as hyperthermia, including the cellular level. Realization of these functions is a characteristic property of medicobiologic "nanoclinics" [19] and nanorobots [23–30].

Investigation of influence of magnetic fluids in vitro, in vivo. In order to estimate the prospects of nanosystems developed for practical use, pre-clinical investigations were started.

It was shown (MTT-test) that magnetic fluids Fe_3O_4/ol.Na and Fe_3O_4/ol.Na/PEG (Fig. 7) with magnetite content $C = 10–50$ μg/ml show practically no cytotoxic activity in vitro on cells of breast cancer line MCF-7.

Cytotoxic activity of the samples of magnetic fluids Fe_3O_4/ol.Na/CP and Fe_3O_4/ol.Na/PEG/CP exceeded cytotoxic activity of the free form of cysplatine in IC_{25} dose for the said cell line.

The investigations were carried out *in vivo* with male mouse hybrids of the line C57B1/6DVA/21, which were inoculated intraperitoneally with ascite carcinoma of Erlich at $6 \cdot 10^6$ cells in an animal. It was shown that the use of Fe_3O_4/ol.Na/PEG/CP nanocomposites as components of magnetic fluids can essentially (by ~40 %) the therapeutic effect of cytostatic preparations.

3. Conclusions

The analysis of the ways of creation of polyfunctional medicobiologic nanocomposites with multilevel hierarchic architecture (that determines their ability to recognition of microbiological objects in biologic media, the targeted delivery and deposition of drugs in target organs, the diagnostics and therapy of diseases on the cellular level), as well as of their physicochemical and medicobiologic properties, shows that such structures can be promising for preparation of new medicinal forms with high efficiency and complex (cytotoxic, immunotherapeutic and hyperthermic) activity.

Further work is planned aimed at optimization of the full technological cycle of production of medicobiological nanocomposites for their use in oncology, completion of preclinical investigations, clarifying the problems related to toxicological aspects, standardization, possibilities of production, etc.

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Нанокомпозити медикобіологічного призначення: реальність і перспективи для онкології

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Проаналізовано шляхи створення поліфункціональних медико-біологічних нанокомпозитів з багаторівневою ієрархічною архітектурою, здатних до розпізнавання мікробіологічних об'єктів у біологічних середовищах, спрямованого транспорту та депонування лікарських препаратів в органах-мішенях, діагностики і терапії захворювань на клітинному рівні. Фізико-хімічні експериментальні дані, медико-біологічні дослідження підтверджують їх перспективність для виготовлення нових лікарських форм онкологічних препаратів комплексної (цитотоксичної, імунотерапевтичної і гіпертермічної) дії.