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NOVEL FUNCTIONAL NANOSCALE COMPOSITES ON THE BASIS OF OLIGOPEROXIDE SURFACTANTS: SYNTHESIS AND BIOMEDICAL APPLICATIONS

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Principal experimental approaches based on tailored synthesis of oligoperoxide surface-active substances and their application for obtaining polymeric and hybrid nanoscale carriers possessing targeted functionality and biocompatibility are presented. Molecular design of novel linear, block and comb-like oligoperoxide surfactants and derived coordinating complexes of transitional and rare earth metal cations is a convenient tool for synthesis of luminescent, magnetic and other functional nanocomposites with controlled size distribution, functionality, reactivity and biocompatibility. The methods developed provide combining the stage of formation of polymeric, metal and metal-oxide nanoparticles with the stage of their surface irreversible modification by functional surface-active oligoperoxides capable of binding physiologically active substances. The availability of reactive ditertiary peroxide fragments provides a possibility for functionalization of developed nanoparticles that were applied for studying phagocytosis, surface markers of pathological cells, and targeted delivery of drugs and antimicrobial remedies.

Key words: oligoperoxide surfactants, metal complexes, radical emulsion polymerization, homogeneous nucleation, functional colloidal particles, «core-shell» polymerization, phagocytosis, drug delivery.

The development of novel nanoparticles and nanocomposites with polymeric shell which provides their colloidal stability with biocompatibility, chemical targeted functionality and reactivity as well as the methods of their synthesis are of a significant interest for the obtaining nanoscale carriers and labels of biomedical application [1–3]. A variety of synthetic technologies for the preparation of nanoparticles of biomedical application with narrowed size distribution and tailored functionality and reactivity are available [1, 2]. At the same time nanoparticle targeted irreversible surface functionalization is one of the main problems of the particle synthesis and biomedical application. As it is referred in [4] «The ability to assemble nanostructures requires precise control of the particle's surface chemistry, where molecules can be coated onto the surface to direct the assembly process. Strategies have been developed to readily permit the modification of a nanoparticle's surface chemistry. In preparing for coatings, the surface of metallic nanoparticles is generally stabilized with a weak ligand that can be easily desorbed from the surface. For other types of nanoparticles where the surface coating with biomolecules may be more difficult, extra processing steps are needed to create a surface with reactive functional groups (–COOH, –SH, or $–NH_2$)». This can lead to novel approaches in treatment of cancer, AIDS and Alzheimer disease.

The creation of theoretical and experimental bases of the synthesis of novel surfactant oligoperoxides (SAP) and coordinative oligoperoxide metal complexes (OMC) on the basis of linear, block and branched structures opens a possibility for obtaining new functional polymeric, inorganic and hybrid colloidal particles [5, 6] by the techniques of sorption modification or/and polymerization. Functional surface-active SAP and OMC are absorbed onto the surface of dispersed polymer and mineral fillers providing the localization of necessary quantity of hydrophobic or hydrophilic and peroxide-containing fragments.

A brief review of principally universal approaches based on tailored synthesis and reactions of functional oligoperoxide surfactants developed in our laboratory for obtaining and application of functionally active

colloids and nanoparticles predominantly for biomedical application is the main goal of our work.

Materials and methods

Oligoperoxide surfactants (SAP) were synthesized on the basis of vinyl acetate (VA), maleic anhydride (MA), and 5-*tert* butylperoxy-5-methyl-1-hexene-3-yne (VEP), styrene (St), methyl methacrylate (MMA), N-vinyl pyrrolidone (N-VP), butyl acrylate (BA), acrylic acid (AA). Polymerization was conducted at 333K in ethyl acetate, using azobisisobutyronitrile (AIBN) as initiator [5]. Surfactant oligoperoxide metal complexes (OMC) were obtained by interaction of functional SAP with copper cations in organic medium at room temperature [6, see also Fig. 1]. Used monomers were purified by double vacuum distillation. MA was purified by vacuum sublimation and after purification its melting point was 325K (ref. [7]). The peroxide monomer VEP was purified by vacuum distillation (active oxygen content was 8.79%; calc. 8.75%). AIBN was purified by re-crystallization from ethanol. Other monomers and solvents (Merck) as well as metal salts were used.

For biological studies nanoparticles of polystyrene and polybutyl-methacrylate structure were synthesized via water dispersion polymerization initiated by OMC or by water dispersion copolymerization of styrene with surface-active monomer (SM). Ammonium persulfate (APS), AIBN, or functional oligoperoxide metal complex (OMC) were used as initiators. Besides, colored latexes were obtained by incorporation of specific dyes (MD) into particles.

The following latexes were used in the study of phagocytosis:

– HM-20 series, polystyrene based polymer:
  - Copolymer styrene: SM 100:10 — APS;
  - Copolymer styrene: SM 100:10 — AIBN;
  - Copolymer styrene: SM 100:1 — AIBN;
  - Copolymer styrene: SM 100:1 — APS.

– Latexes with incorporated dye:
  - Copolymer styrene: SM 100:10 + 10% MD, initiator — APS (Viola-1);
  - Copolymer styrene: SM 100:10 + 10% MD, initiator — APS, T=70°C (Viola-2);
  - Copolymer styrene: SM 100:10 + 20% MD, initiator — APS (Viola-3).

– Polyacrylate based latexes:
  - Butyl methacrylate: SM 100:10 +20% MD, initiator — APS, T=80 °C (Viola-4);
  - Butyl methacrylate: methyl methacrylate + glycidyl methacrylate, initiator — OMC.

Fractionation of nanoparticle suspensions by differential centrifugation

For obtaining monodisperse latexes based on styrene and acrylate polymers the following scheme of differential centrifugation was applied (Fig. 2). The obtained fractions I, II, III were stored as 10% (v/v) water suspensions in the presence of 0.02% sodium azide, as preservative.

Fractionation of metallic nickel particles coated with polymer was performed by spontaneous sedimentation for different time intervals. The sedimentation rate at 1 g was high enough without centrifugation. The suspension was submitted to ultrasonic treatment for effective disintegration (dispersion), the liquid in a vessel was kept of constant level 7 cm, fraction of particles which sediment between 4 and 7 min was collected. The procedure was repeated three times.

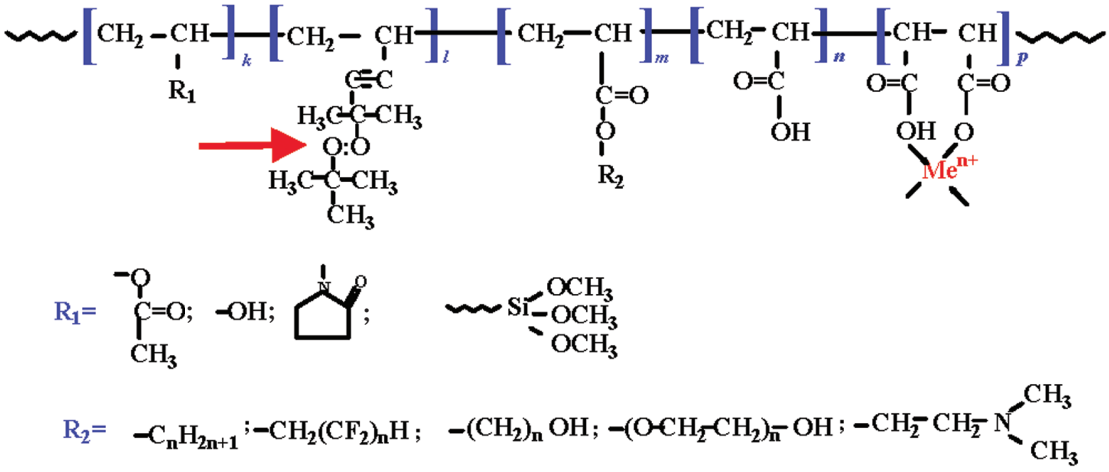

Fig. 1. **Surface-active oligoperoxide polymeric complexes with metal cations. Radicals R_1 and R_2 are presented. Oligoperoxide group is noted with red arrow**

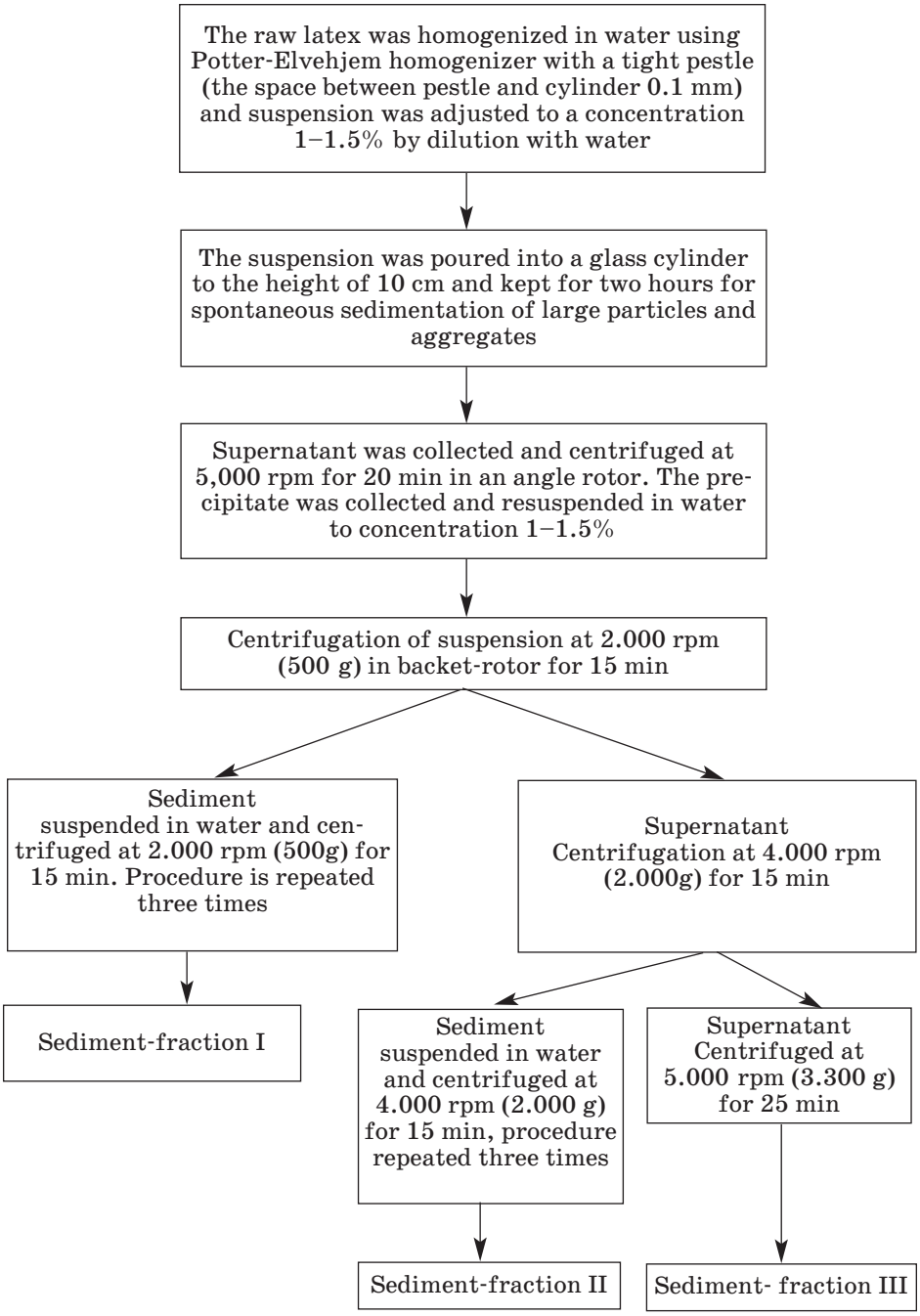

Fig. 2. Scheme of differential centrifugation of monodisperse latexes based on styrene and acrylate polymers

For light microscopy and micrometry of particle fractions, the smears were prepared on microscope slides as follows: 50 μl of suspension was centrifuged in Eppendorf' tube, supernatant was discarded, the sediment was suspended in two volumes of bovine serum and smears were obtained as conventionally accepted in hematology. Microscopic investigation was conducted using objective ×40 and ocular ×15. The size of particle was measured using an ocular-micrometer AM9–2, 200–300 particles were measured in 3–4 fields and mean diameter was calculated.

Functionalization of nanoparticles with specific proteins, opsonization of nanoparticles for phagocytosis

Opsonization of latexes was performed as following. Aliquot of latex was centrifuged, the sediment of particles was washed twice with 0.1 M pyrophosphate buffer pH 8.9 and

was suspended in double volume of human serum or 0.5% solution of concanavalin A in the same buffer. Suspension was kept in refrigerator for 24 hours, thereafter washed several times with PBS and the final suspension was standardized according to the number of particles per ml (usually 400–500 mln/ml).

Isolation of white blood cells

Phagocytosis test was performed on leukocyte suspension of human blood, which was prepared as follows. In a sterile plastic syringe 1–2 ml of blood was taken with anticoagulant heparin (0.1 volume of medical heparin solution diluted 1:10 with sterile saline). Thereafter 5% sterile solution of dextrane in saline was aspirated up to the final concentration of dextrane 1%, mixed and the syringe at an angle 45° for 20–30 min. In the presence was placed of dextrane, the sedimentation of red blood cells is accelerated, leaving over erythrocytes plasma with leukocytes and small residue of erythrocytes. The volume of plasma enriched with leukocytes is about half of initial blood volume. It was carefully transferred from syringe through plastic capillary tube into sterile Eppendorf's plastic tube. In obtained leukocyte suspension the number of granulocytes and lymphocytes was determined by the following way: 20 μl of leukocyte suspension was taken in an Eppedorf's tube and cells were pelleted by centrifugation for 2 min at 1.500 rpm. Plasma was carefully discarded and cells were suspended in 40 μl solution of 1% acetic acid with 0.5 % methyl green. After 5 min the suspension was investigated in hemocytometric unit (Horyaev type) at a magnification ×600 and nuclei of mononuclears and polymorphonuclears were differentially counted. The number of polymorphonuclears (PMN) and mononuclears (MN) in 1 μl were calculated.

As traditional object of phagocytosis the killed cells of yeast *Debariomyces hansenii* were used. The suspension was prepared by dispersion of 1 mg of powder of dried cells in 0.5 ml of water. Effective dispersion was achieved by multiple passing of suspension through the finest needle of insulin syringe. The concentration of particles in suspension was determined by counting of aliquot diluted 1:50 with water in hemocytometric unit at magnification ×600. Usually the concentration 2 mg/ml corresponds to 130–150 mln of yeast cells per ml. Nevertheless, each series of suspension should be standardized. For a prolong storage (two weeks) suspension is preserved with 0.02% of sodium azide.

Experimental procedure of phagocytosis test

1. 50 μl of leukocyte suspension is placed in plastic Eppendorf tube, thereafter 1 μl of particle suspension is added, mixed and incubated at 37 °C for 50–55 min.

2. Tubes are centrifuged at 1.500 rpm for 2 min, supernatants are carefully aspirated leaving 3–4 μl, in which the precipitated cells are suspended and smears are prepared on microscope slides. Smears are fixed in methanol and stained by Romanovsky-Giemsa method.

3. Smears are investigated by light microscopy with the use of immersion optic (×1.350). More then 500 cells in several fields are registered and number of ingested particles (yeast cells or polymer beads) in each cell is noted (including zero). The next indexes of phagocytosis are calculated: phagocytosis indices, phagocytosis number I (extensive) and phagocytosis number II (intensive), defined as follows:

Phagocytosis index — percent of phagocyting cells in cell population;

Phagocytosis number I (extensive) — amount of ingested particles, calculated for all registered cells;

Phagocytosis number II (intensive) — amount of ingested particles, calculated on phagocytic (active) cells only.

Results and Discussion

Among a variety of methods of preparing of functional polymeric particles, the methods based on using SAP and OMC as universal and convenient tool are looking to be the most prospective for obtaining of reactive polymeric particles of tailored size and functionality.

1. Water dispersion polymerization initiated by OMC

Previously we have studied the formation of primary reactive polymer nanoparticles with functional shell by the technique of water dispersion polymerization initiated by OMC [8, 9]. The main regularities of controlled radical polymerization initiated by oligoperoxide Me^{n+}-containing surfactants indicate the possibility of the obtaining polymer water dispersions comprising of unimodal nanoparticles with particle size in the range 30–70 nm as shown on Fig. 3 reactive functional shell capable to radical, condensation and other reactions [9] in accordance with the scheme of polymerization presented below (Fig. 4).

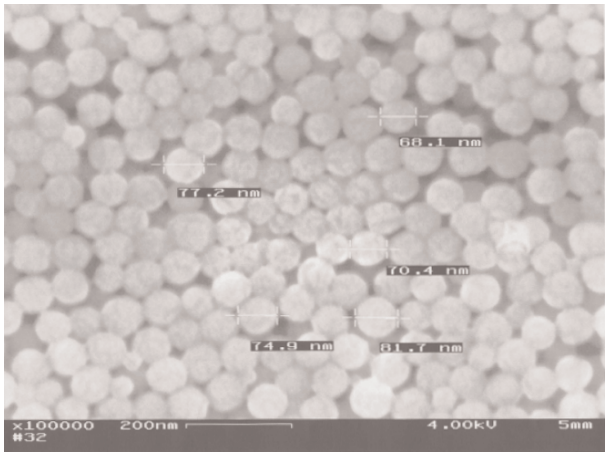

Fig. 3. SEM picture of functional polystyrene nanoparticles

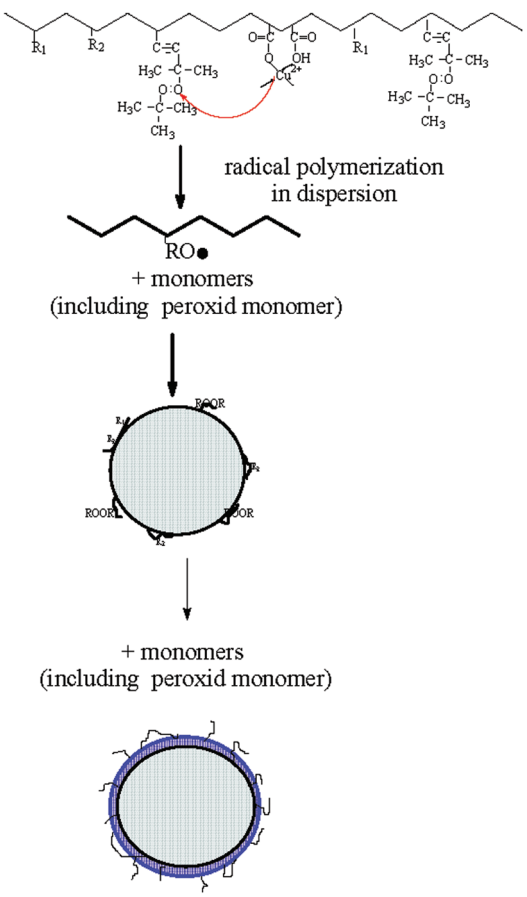

Fig. 4. Assumed mechanism of functional polymeric nanoparticle formation as a result of styrene water dispersion polymerization initiated by OMC

Using coordinating oligoperoxide complexes with cations of rare earth elements for the initiation of styrene dispersion polymerization provides obtaining luminescent polymeric nanoparticles with narrowed particle size distribution (Fig. 5 and 6). The nanoparticle luminescent properties were investigated by professor A. Voloshinovsky.

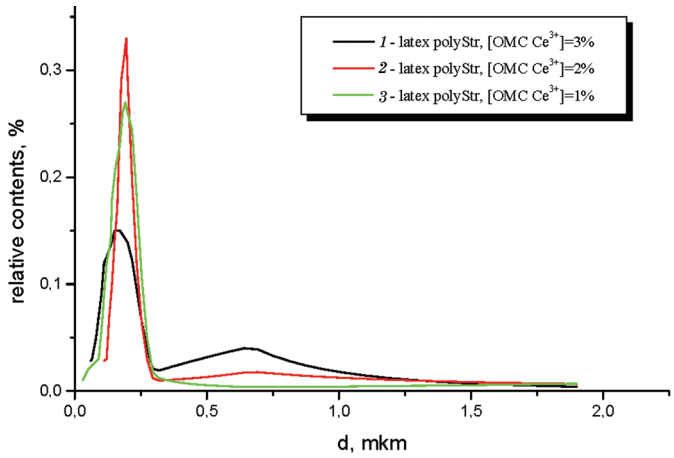

Fig. 5. Size distribution of luminescent polymeric nanoparticles:
1— polystyrene latex with 3% of Ce^{3+} complex;
2 — polystyrene latex with 2% of Ce^{3+} complex;
3 — polystyrene latex with 1% of Ce^{3+} complex
($[Ce^{3+}]$ =1.25%)

One can see that highly monodisperse nanoparticles can be synthesized only at optimal content of luminescent OMC in water dispersion system as initiator and stabilizer. This is explained, possibly, by the change of nanoparticle formation mechanism at different OMC concentrations.

Functional polystyrene nanoparticles possess intense luminescent ability due to availability of coordinated Ce^{3+} cations in the functional particle shell. It is evident that decrease of nanoparticles concentration in water system leads to the enhancement of luminescence intensity as a result of the increase of the system transparency.

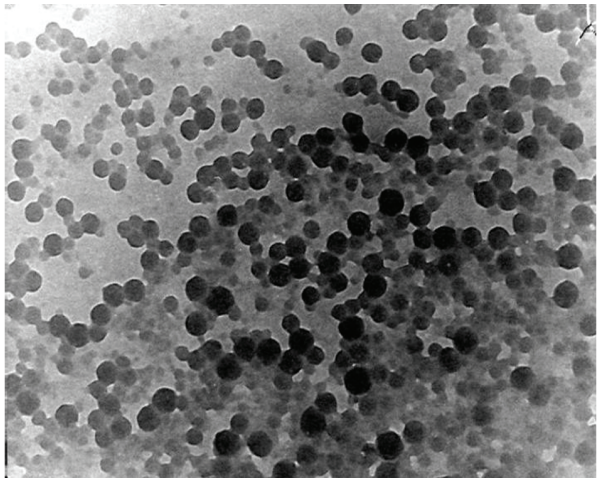

Fig. 6. TEM picture of polystyrene nanoparticles with functional shell containing coordinated Ce^{+3} cations:
2% of Ce-oligoperoxide complex per H_2O
($[Ce^{3+}]$ =1.25%)

As a result of sorption immobilization of luminescent surface-active oligoperoxide modifiers onto magnetic ferric oxide nanoparticle surface the novel functional magnetic and luminescent nanoparticles were synthesized (Fig. 7).

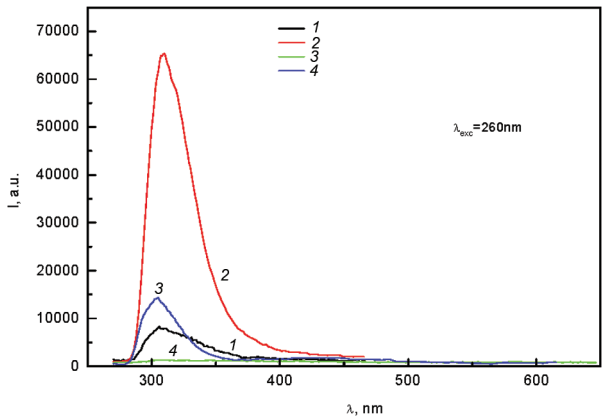

Fig. 7. Spectra of luminescence of coordinating oligoperoxide Ce^{3+} complex, polymeric (*1*, *2*, *3*) and hybrid magnetic (*4*) nanoparticles with functional shell containing oligoperoxide Ce-complex:

1 — Water dispersions of polystyrene nanoparticles with functional shell containing coordinated Ce^{3+} cations (3% of Ce-oligoperoxide complex per H_2O ($[Ce^{3+}] = 1.25\%$, [SC] latex = 17.0%);

2 — Water dispersions of polystyrene nanoparticles with functional shell containing coordinated Ce cations (3% of Ce-oligoperoxide complex per H_2O ($[Ce^{3+}] = 1.25\%$, [SC] latex = 4.25%);

3 — Water dispersions of polystyrene nanoparticles with functional shell containing coordinated Ce cations (3% of Ce-oligoperoxide complex per H_2O ($[Ce^{3+}] = 0.03\%$, [SC] latex = 17.0%);

4 — Fe_3O_4 nanoparticles with functional shell containing coordinated Ce^{3+} cations (Ce-oligoperoxide complex $[Ce^{3+}] = 1.25\%$)

2. Water dispersion co-polymerization with surface-active monomers (SM and MD, have been synthesized by dr. O. Hevus and prof. V. Novikov respectively, Lviv Polytechnic National University)

Novel monodisperse functional and colored polymer water dispersions with definite particle size and functionality were developed using surface-active functional maleates (SM) and acrylates containing chromophore fragments (MD) as comonomers at water dispersion emulsifier free copolymerization of styrene. The structures of used SM are presented on the Fig 8.

It is evident from the Fig. 9 that copolymerization of styrene with SM and MD of above-mentioned structures provides obtaining controlled amount of highly monodisperse colored nanoparticles with size 300 nm depending on the content of SM in initial monomer system.

3. Seeded polymerization initiated from the surface of the particles modified by OMC

Functional polymeric nanoparticles containing radical forming sites in oligoperoxide shell immobilized on the particle surface are efficient initiators of seeded polymerization providing grafting of various functional chains at the definite distance from the particle core. The experimental results of seeded low temperature polymerization initiated from the surface of functional polymer nanoparticles presented in the Table 1 display he formation of composite particles with «core-shell» morphology and the possibility of obtaining multi-layer reactive shell ws a result. It is evident (Fig. 10) that various monomers and monomer systems can be used for the

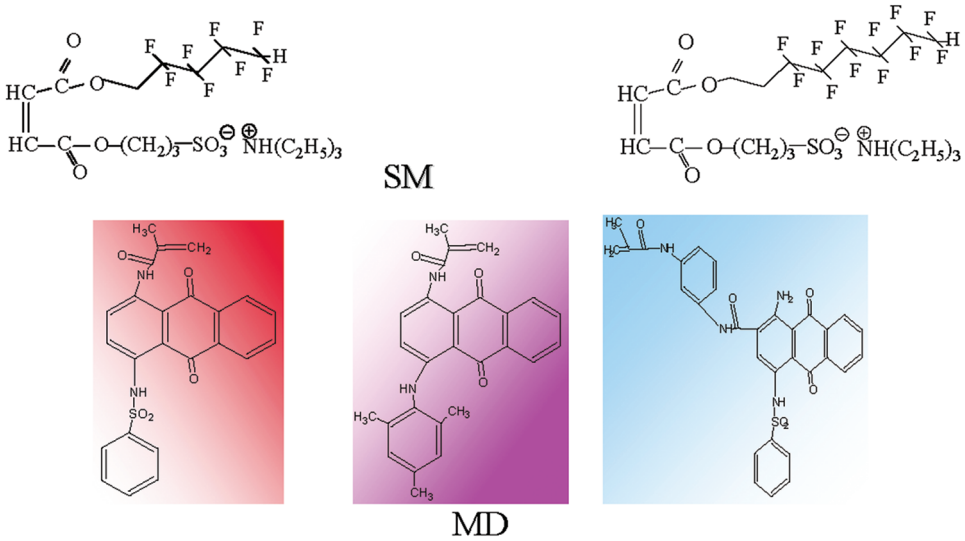

Fig. 8. Structures of surface-active monomers (SM) and monomeric dyes (MD) used for the obtaining colored polymeric nanoparticles

Table 1. **Multi-stage seeded (co) polymerization initiated from the particle surface (293K)**

First stage			Second stage*				Third stage **			
Latex particle structure	Dry residue, %	D part., μm	Monomer for the second shell***	Dry residue, %	D part., μm	Polymerization rate, W,%/h	Monomer for the third shell	Dry residue, %	D part., μm	Polymerization rate, W,%/h
Core St-BA-VEP 53:32:15Shell OMC	22.0	0.015	F-MA Si-MA BA-GMA 90:10	27.0 25.5 28.0	0.020 0.018 0.020	7.2 9.0 10.2	– – –	– – –	– – –	– – –
Core St-BA-AA 70:25:5Shell — OMC	23.0	0.010	VEP-BA 50:50	29.0	0.014	9.0	Si-MA F-MA	33.0 32.2	0.016 0.016	7.2 9.0

* The formation of the second shell was initiated by residual peroxide OMC in the particle shell.
** The formation of the third shell was initiated by additional OMC sorbed onto particle surface (0.5% per monomers).
*** F-MA-2,2,3,3-tetrafluoropropyl-2-methacrylate; Si-MA — (3-trimethoxysilyl) propyl-2-methacrylate; GMA — (2,3-epoxy propyl)-methacrylate.

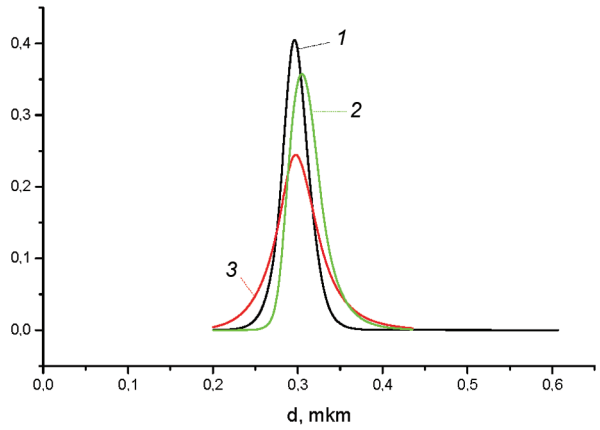

Fig. 9. Colored polystyrene nanoparticle size distribution in water dispersion systems synthesized with various content of surface-active maleates:
1 — 20% of SM St;
2 — 10% of SM St;
3 — 5 % SM St

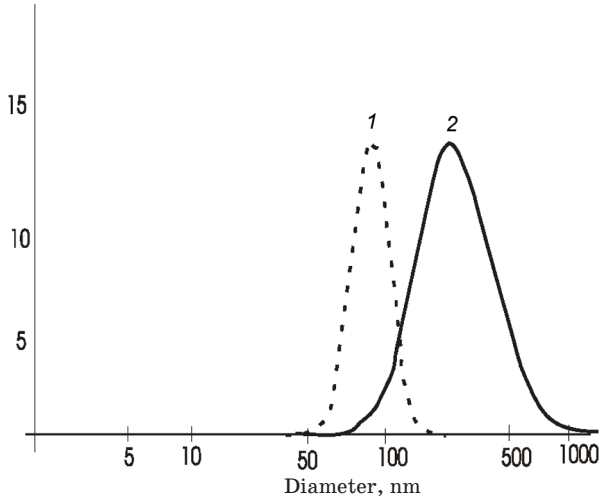

Fig. 10. Polystyrene nanoparticle size distribution after OMC initiated (*1*) and seeded (*2*) water dispersion polymerization

fomation of the second and third functional polymer shells on the particles resulting in the change of particles hydrophobic-hydrophilic properties, their functionality and enhancement of size.

The dependences of seeded polymerization rate on the concentration of sodium pentadecyl sulphonate and particle-initiator content in the systems testify to the occurrence of the polymerization exceptionally on the primary particle surface providing the increase of their size. No news particles are formed during seeded polymerization initiated by particles modified by OMC.

As a result of seeded water dispersion polymerization initiated by radical forming sites in functional oligoperoxide shell new particles with complicated morphology and targeted functionality, compatibility (including biocompatibility) and reactivity can be synthesized.

4. Homogeneous nucleation from the salt solutions in the presence of SAP or OMC

This is the method of the formation of primary reactive inorganic particles with functional shell by homogeneous nucleation from the solutions of corresponding metal salts in the presence of SAP and OMC [10–12] as it follows from the scheme (Fig. 11).

Reactive Ni colloids, Fe_3O_4, Ag and other nanoparticles with narrowed particle size distribution and tailored functional shell and compatibility were synthesized by this technique in the presence of oligoperoxide surfactants [13–17]. The number average particle size distribution testifies to the tendency of the formation of unimodal nanoparticles at their formation in the presence of OMC sur-

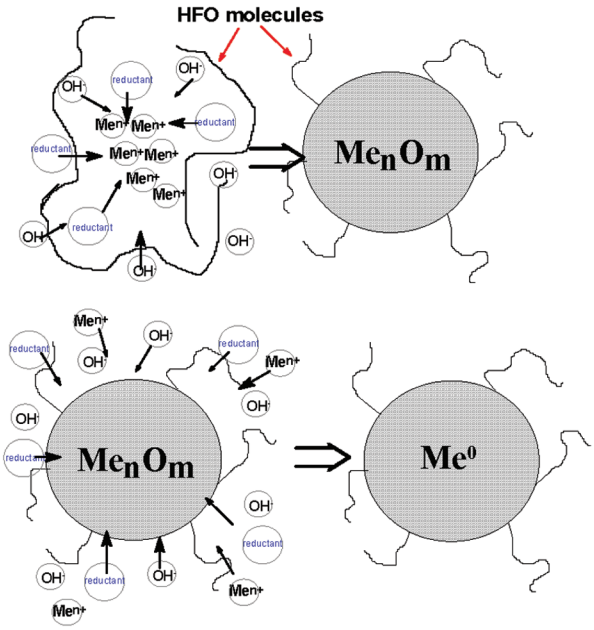

Fig. 11. Scheme of homogeneous nucleation of metal and metal oxide particles with functional OMC shell

factant (Fig. 12). This is explained as we have shown earlier [8–10] by the displacement of the reaction of particle nucleation into micelle-like structures formed by SAP, which are the templates determining the particle size.

TEM (Fig. 12) study of nanoparticle hydrosols witness the possibility of controlled synthesis of nanoparticles with size in the range 5–20 nm depending the nature and content of SAP and conditions of corresponding salt reduction in the presence of SAP as template and stabilizer.

SAXS technique (Table 2), TEM (Fig. 12), SEM microscopy and magnetic measurements testify to favor of the realization of more complicated mechanism of ferric oxide formation as a result of homogeneous nucleation in the presence of functional oligoperoxide surfactants in accordance with scheme (Fig. 13).

Proceeding from SEM and TEM microscopy and SAXS analysis it can be suggested that integral Fe_3O_4 particles with size near 100–150 nm comprise of magnetic mineral core containing nanocrystals with size 8–12 nm and functional

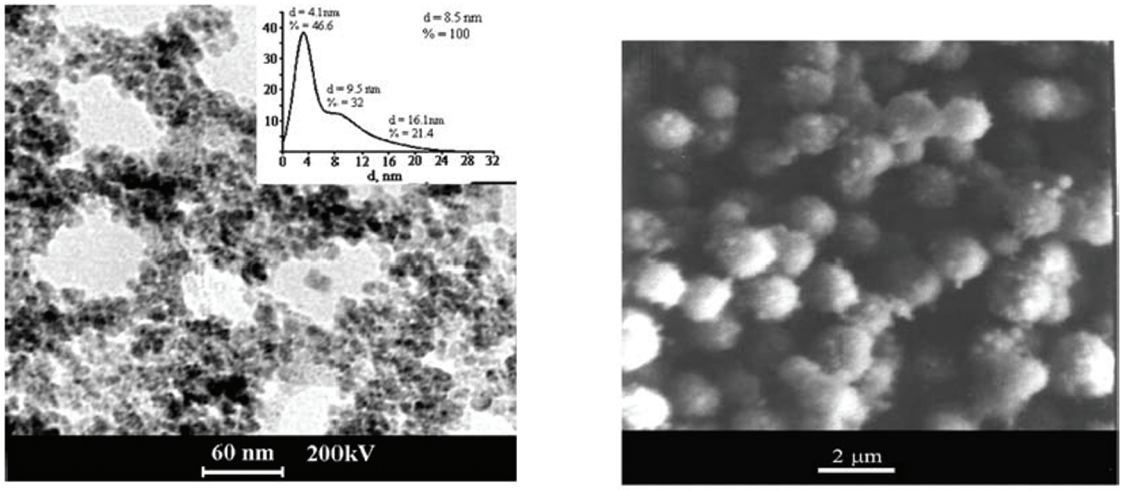

Fig. 12. Micrographs of functional hybrid nanoparticles: *a* — TEM image of magnetite crystals (inset: number average distribution of crystal size obtained from SAXS data); *b* — SEM image of colloidal nickel particles

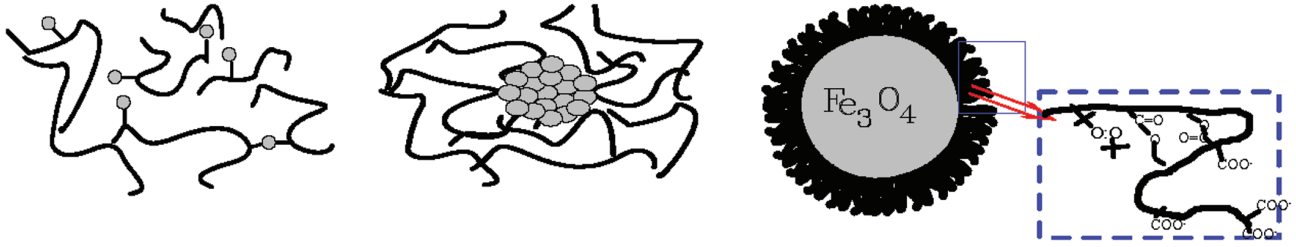

Fig. 13. Synthesis and transformations of biocompatible functional nanocarriers on the basis of Fe_3O_4

Table 2. **The influence of the synthesis temperature and SAP concentration on the characteristics of functional magnetite nanoparticles**

T, K	[SAP], %	Crystal size, d_{cr}, nm	Seed concentration $N_s$mole/l·10^6	SAP molecule amount per 1 seed N_{SAP}·10^3	Polymer-mineral particle size d_N, nm* / weight mode, %	Polydispersity index k	Seed amount per 1 particle N·10^{-3}
293	0	10.4±1.0	1.09	0	146±57/ 96.5	1.66	2.77
	0.2	9.6±0.9	1.38	0.28	97±27 / 92.1	4.62	1.03
	2	8.9±0.9	1.74	2.21	78±26 / 78.4	2.94	0.67
333	0	12.6±1.1	0.61	0	71±21 / 82.4	3.19	0.18
	0.2	10.5±1.0	1.06	0.36	67±19 / 73.3	2.21	0.26
	2	9.6±0.9	1.38	2.78	–	–	–
363	0	13.3±1.2	0.52	0	87±39 / 59.3	1.83	0.28
	0.2	12.4±1.1	0.64	0.60	72±27 / 44.6	1.66	0.13
	2	10.2±1.0	1.15	3.33	69±27 / 58.1	2.14	0.47

* Number average diameter calculated for the mode with maximum weight in particle diameter distribution plot.

polymeric shell providing radical reactions initiated from the particle surface and other polymer analogous transformations.

SAP concentration and temperature of the synthesis are main factors defining integral nanoparticle size as well as the size of nanocrystals (Table 2).

TEM micrographs of magnetite nanoparticles after 4 and 40 s sonification confirm complicated Fe_3O_4 nanoparticle morphology (Fig. 14).

The availability of reactive functional shell on the nanoparticle surface not only provides tailored rheological characteristics and compatibility with various media but also the possibility of the occurring of radical and other reactions with the participation of functional fragments located in the particle shell on the definite distance from the core.

5. Seeded polymerization initiated from inorganic particle surface modified by OMC

The presence of radical-forming sites on the particle surface causes the possibility of low temperature radical formation by ditertiary peroxide groups and grafting polymer chains to surface with the formation of new functional shell at given distance from the surface (Fig.15 and Table 3).

One can see (Fig. 15) that seeded polymerization initiated from the surface of inorganic particles obeys the same regularities, which are peculiar to the polymerization initiated from the polymer particle surface, namely, independence on the concentration of the additional emulsifier. This proves the occurrence of graft polymerization only on the particle surface and the impossibility of particle formation in the solution. The polymerization rate and conversion depend strongly on the modified filler nature and its content in the reaction system. The study of the particles after seeded polymerization witnesses about the increase of their size and respectively tailored formation of grafted chains containing the definite amount of active epoxide and peroxide fragments.

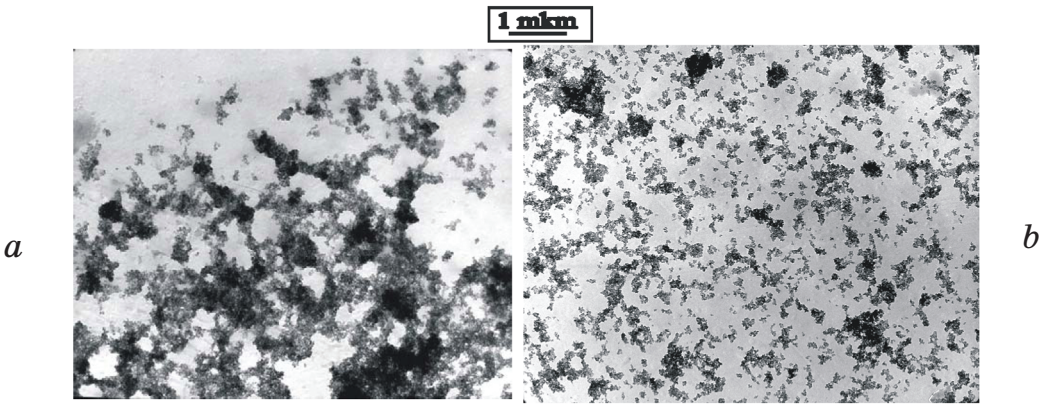

Fig. 14. **TEM pictures of Fe_3O_4 nanoparticles after 4s (*a*) and 40s (*b*) ultrasound treatment**

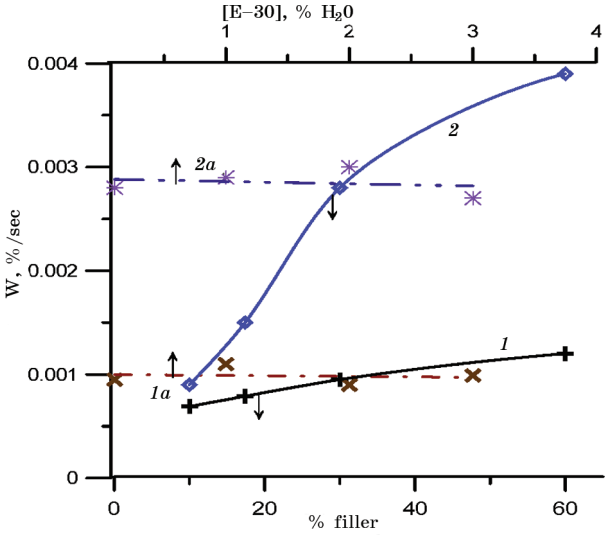

Fig. 15. **Water dispersion polymerization rate of VEP-GMA*-St mixture vs. filler content (*1*, *2*) and concentration of emulsifier E-30 (*1a*, *2a*).**
Initiation from the OMC modified filler surface: g-Fe_2O_3 ([OMC] = 0.7%) (*1*, *1a*) colloidal Ni particles ([OMC] = 0.45%) (*2*, *2a*); 291 K

* Glycidil methacrylate.

phagocytic cells (neutrophile granulocytes or macrophages) depends upon the properties of particle surface, especially upon surface proteins. Substances, including proteins, which stimulate phagocytosis are called «opsonins» and process of coating particles with these substances does «opsonization». The most effective opsonins are immunoglobulins and antibodies, that are adsorbed on particle surface by different bonds (physical or chemical).

In our experiments, opsonization of microparticles was performed by using proteins of human blood serum, or concanavalin A. The last one is a plant protein — lectin, which selectively binds carbohydrates mannose, glucose and acetamidoglucose. In natural conditions, it binds with polysaccharides glucans and mannans, or with glycoproteins, exposing residues of mannose and acetamidoglucose. Being absorbed on particle surface, concanavalin A favors the attachment of particle to cell surface of phagocytes ant its ingestion.

Investigations of phagocytosis activity obtained with different types of latexes are shown in table 4.

Table 3. **Characteristics of copolymer GMA-VEP-St grafted to the surface of inorganic nanoparticles (291 K; monomers: H_2O = 1:5; GMA-VEP-St 2:1:1)**

Particles	Particle content,%	Content of grafted copolymer, %	Composition of grafted copolymer, %		
			GMA	VEP	St
γ-Fe_2O_3 [OMC] = 0.7%	17.4	1.6	50.0	25.0	25.0
	30	2.5	60.0	19.0	21.0
	60	5.0	75.0	16.0	9.0
Colloidal Ni [OMC] = 0.45%	17.4	0.8	65.0	5.5	29.5
	30	1.1	55.0	6.0	39.0
	60	2.5	50.0	10.0	40.0

6. Potentials for biomedical application: Employment of nanoparticles in study of phagocytic activity of human blood granulocytes

The characteristics of different particle fractions obtained by differential centrifugation are presented in Fig. 16. The most suitable for application in phagocytosis studies were dimension and homogeneity of fractions III of styrene polymers, which were sedimented at relatively high g-values. The diameter of particles in these suspensions was in 0.7–1.5 μm range.

Latex particles as objects of phagocytosis were pretreated with different proteins, which is defined as «opsonization». It is known, that efficiency of ingestion of particles by

In Fig. 17 an example of cytological pattern of phagocytosis with different objects including polymer coated nickel micro particles is presented.

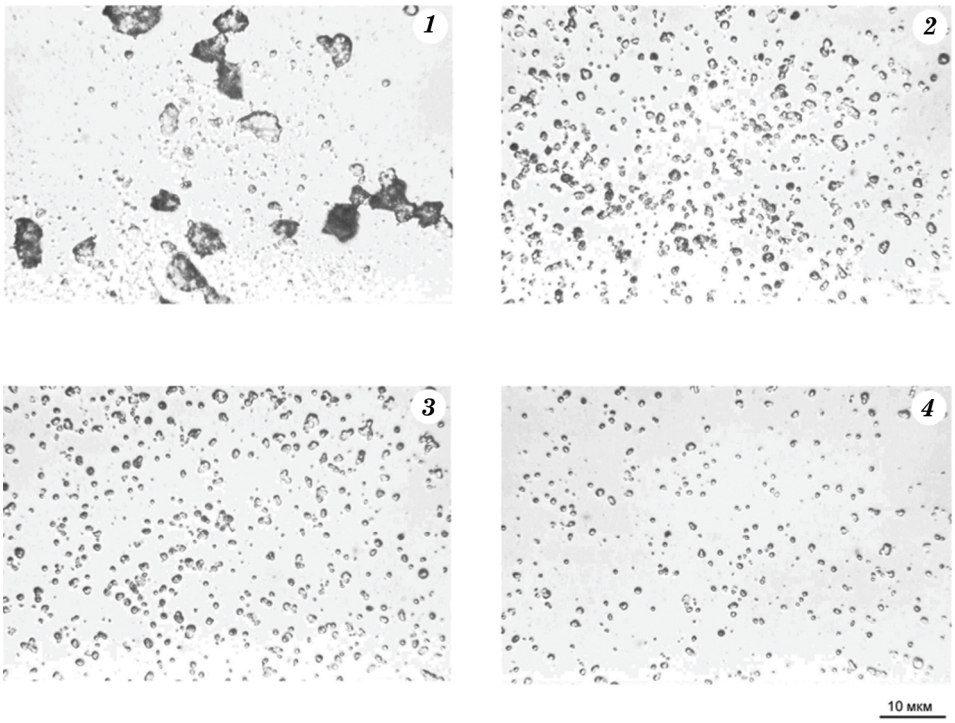

Fig. 16. **Fractions of nanoparticles obtained with centrifugation of latex at different g-values:**
1 — sediment, obtained by spontaneous sedimentation (1g) for two hours;
2 — fraction, obtained by centrifugation at 540 g for 15 min;
3 — fraction, obtained by centrifugation at 2.000 g for 15 min;
4 — fraction, obtained by centrifugation at 3.300 g for 25 min

Table 4. **Effectiveness of polystyrene latexes as objects of phagocytosis**

N	Object of phagocytosis	Phagocytosis index (%)	Phagocytosis number I (extensive)	Phagocytosis number II (intensive)
1	Yeast cells *Debariomyces hansenii*	40	0.65	1.6
2	Unsensibilized latex #1	18	0.2	1.3
3	Latex #1	43	1.2	2.7
4	Latex #1, sensibilized with proteins of human blood serum	50	1.5	3.0
5	Latex #3, sensibilized with concanavalin A	34	0.9	2.7
6	Latex #3, sensibilized with proteins of human blood serum	45	1.0	2.4
7	Latex NM-20, sensibilized with concanavalin A	21	0.3	1.3
8	Latex NM-20, sensibilized with proteins of human blood serum	17	0.2	1.1
9	Latex #11, sensibilized with concanavalin A	47	0.9	1.9
10	Latex #11, sensibilized with proteins of human blood serum	65	1.9	2.9
11	Latex Viola-1 (LV-1), sensibilized with concanavalin A	40	0.9	2.4
12	Latex Viola-2 (LV-2), sensibilized with concanavalin A	47	0.9	2.4
13	Latex Viola-3 (LV-3), sensibilized with concanavalin A	50	1.0	2.0

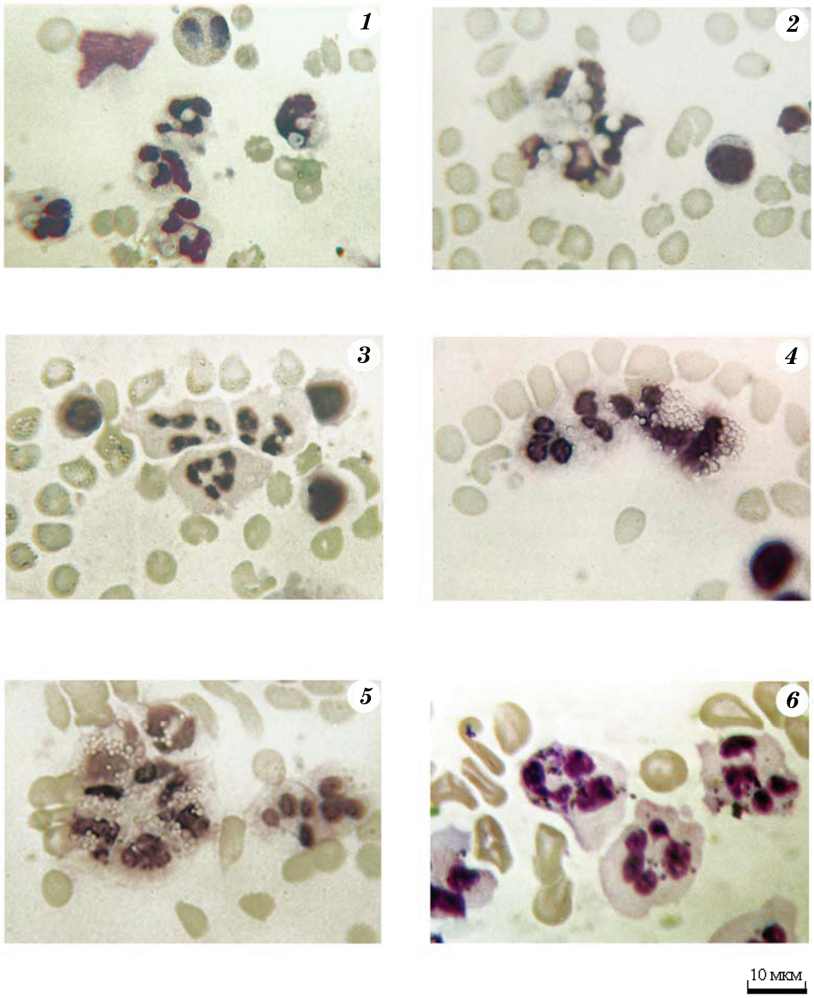

Fig. 17. Activity of polymorphonuclear leukocytes with different objects of phagocytosis:

1, *2* — phagocytosis of commonly used object — yeast cells Deberiomyces hansenii.
1 — in the central part four moderately active neutrophiles (1–2 yeast cells ingested) are visible, in upper part (12 o'clock) nonphagocyting eosinophile (common state), in the left part of image (7 o'clock) — active monocyte;
2 — the group of hyperreactive neutrophiles, the cytoplasm is completely occupied with ingested yeasts;
3, *4*, *5* — particles of latex NM-20 as objects of phagocytosis;
3 — opsonization with proteins of human blood serum, two silent and one weak activity neutrophiles are present, on periphery there are three silent (non-phagocytic) lymphocytes (common state);
4 — opsonization with proteins of human blood serum, the group of hyperreactive neutrophiles;
5 — opsonization with concanavalin A; in the left side hyperreactive neutrophiles are visible, in the right side — two silent neutrophiles;
6 — nickel particles coated with polymeric envelope as an object of phagocytosis

In this research various approaches of oligoperoxide metal complexes and surface-active oligoperoxides use for the activation of colloidal particles causing polymer grafting onto their surface have been demonstrated. They permit to obtain nanoparticles of «core-shell» structure with fragments providing their tailored compatibility, functionality and reactivity including biocompatibility and specific biological activity.

Functional nanoparticles including magnetic, colored and luminescent ones contain the spacers with the functional groups, which are capable to radical and polymer-analogous transformations. They were successfully tested as stained or magnetic labels for investigation of phagocytosis, labeling of pathological cells as well as nanocarriers for addressed drug delivery.

In phagocytosis experiments, the advantage of latex microparticles as compared with yeast cells is the existence of chemically conditioned surface and possibility of its modification by attachment of distinct ligand with known

structure. The surface of yeast cells is very complex and manifold, dependent from the strain of microorganism. Due to this consideration, the interpretation of results obtained with using microparticles is much easier.

Efficiency of ingestion of latex particles by phagocytes depends from chemical nature of polymer, technology of production, opsonization. Opsonization with proteins of blood serum or with lectins (in our case with concanavalin A) significantly increases the efficiency of their ingestion by phagocytes and achieves values for classical object of phagocytosis — yeast cells.

Nickel microparticles coated with polymer envelope and opsonized with blood serum proteins are comparatively well ingested by phagocytes and can be used for isolation of phagocyting cells from the rest of population using magnet, as particles possess ferromagnetic properties.

Proposed technology of synthesis of latexes and their fractionation for obtaining a monodisperse suspensions can be used for determination of phagocytosis activity of blood cells in clinical laboratories with diagnostic purpose.

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НОВІ ФУНКЦІОНАЛЬНІ НАНОРОЗМІРНІ КОМПОЗИТИ НА ОСНОВІ ОЛІГОПЕРОКСИДНИХ СУРФАКТАНТІВ: СИНТЕЗ І ЗАСТОСУВАННЯ В БІОЛОГІЇ ТА МЕДИЦИНІ

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Розглянуто основні експериментальні підходи до цілеспрямованого синтезу поверхнево-активних олігопероксидних речовин і їх застосування з метою одержання полімерних і гібридних нанорозмірних носіїв, яким притаманна скерована функціональність та біосумісність. Конструювання нових лінійних, блокових та гребенеподібних олігопероксидних сурфактантів, а також їхніх похідних координаційних комплексів із катіонами перехідних та рідкоземельних металів є зручним інструментом для синтезу люмінесцентних, магнітних та інших функціональних нанокомпозитів із регульованим розподілом за розмірами, функціональністю, реактивністю та біосумісністю. Розроблені методи дозволяють поєднати стадію формування полімерних, металевих та металооксидних наночастинок зі стадією необоротної модифікації їхньої поверхні функціональними поверхнево-активними олігопероксидами, здатними зв'язувати фізіологічно активні речовини. Наявність реакційноздатних дитретинних пероксидних фрагментів уможливлює функціоналізацію створених наночастинок, що їх було застосовано для дослідження фагоцитозу як поверхневі маркери патологічних клітин, а також для спрямованого доставлення лікарських препаратів.

Ключові слова: олігопероксидні сурфактанти, металеві комплекси, радикальна емульсійна полімеризація, гомогенна нуклеація, функціональні колоїдні частинки, затравкова полімеризація, фагоцитоз, доставлення лікарських препаратів.

НОВЫЕ ФУНКЦИОНАЛЬНЫЕ НАНОРАЗМЕРНЫЕ КОМПОЗИТЫ НА ОСНОВЕ ОЛИГОПЕРОКСИДНЫХ СУРФАКТАНТОВ: СИНТЕЗ И ПРИМЕНЕНИЕ В БИОЛОГИИ И МЕДИЦИНЕ

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Рассмотрены основные экспериментальные подходы к целенаправленному синтезу олигопероксидных поверхностно-активных веществ и их использованию для получения полимерных и гибридных наноразмерных носителей, обладающих заданной функциональностью и биосовместимостью. Конструирование новых линейных, блочных и гребеневидных олигопероксидных сурфактантов, а также их производных координационных комплексов с катионами переходных и редкоземельных металлов является удобным инструментом для синтеза люминесцентных, магнитных и других функциональных нанокомпозитов с регулируемым распределением по размеру, функциональности, реакционной способности и биосовместимости. Разработанные методы обеспечивают совмещение стадии формирования полимерных, металлических и металлооксидных наночастиц со стадией необратимой модификации их поверхности функциональными поверхностно-активными олигопероксидами, способными к связыванию физиологически активных веществ. Наличие реакционноспособных дитретичных пероксидных фрагментов обеспечивает возможность функционализации полученных наночастиц, которые использовались для изучения фагоцитоза как поверхностные маркеры патологических клеток, а также для целевой доставки лекарственных препаратов.

Ключевые слова: олигопероксидные сурфактанты, металлические комплексы, радикальная эмульсионная полимеризация, гомогенная нуклеация, функциональные коллоидные частицы, затравочная полимеризация, фагоцитоз, доставка лекарственных препаратов.