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FUNCTIONAL OLIGOPEROXIDE BASED LUMINESCENT POLYMER AND POLYMER-MINERAL NANOCOMPOSITES

A. Zaichenko

zaichenk@polynet.lviv.ua

Lviv Polytechnic National University

The main aim of the study was:

tailored synthesis of novel functional polymer and mineral colloids and nanoparticles with magnetic, luminescent and scintillation properties on the basis of oligoperoxide surfactants and derived coordinated metal complexes for biomedical application

Talk outline

1. Introduction. Functional reactive surface-active oligoperoxides and derived oligoelectrolyte and non ionic surfactants of block, comb-like or branched structures.

2. Luminescent, coloured and magnetic nanocomposites. The synthesis, functionalization and characterization.

3. Cellular studies and potential biomedical application. Cell detection, tagging and treatment.

1. Introduction. Functional reactive surface – active oligoperoxides and derived oligoelectrolytes and PEGylated oligomers of linear, block and comb-like structures.

The main approaches of tailored synthesis of functional oligoperoxides and derived polymers

1. Co polymerization of unsaturated ditertiary peroxides with functional monomers in hydrocarbon media.

2. Telomerization of functional monomers in the presence of peroxide-containing telogen in hydrocarbon media.

3. Emulsifier free water dispersion co polymerization of functional monomers with unsaturated ditertiary peroxide or peroxide-containing telogen participation.

4. Polymer analogous transformations using carboxyl, amino, epoxy, isocyanate, anhydride and other reactive functional groups of peroxide-containing oligoelectrolytes.

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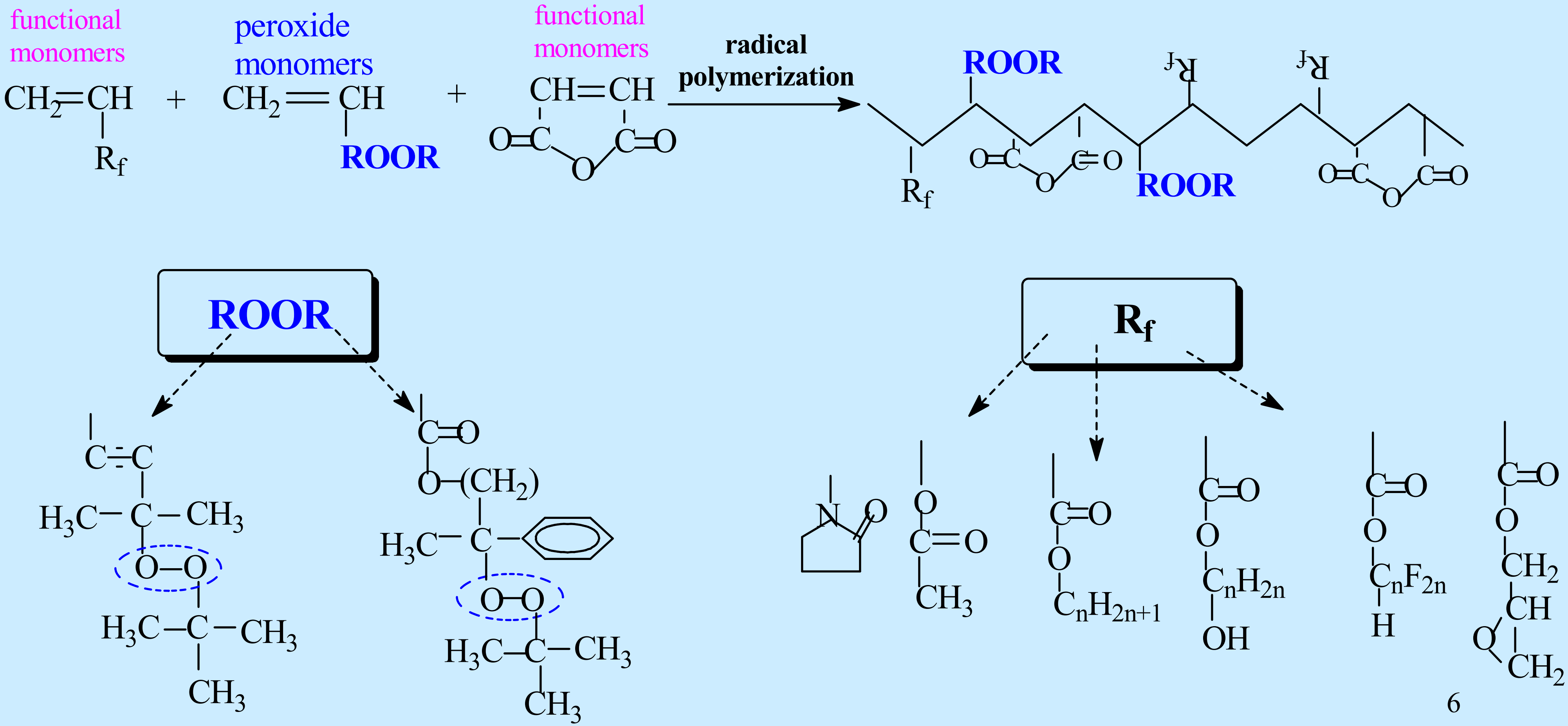
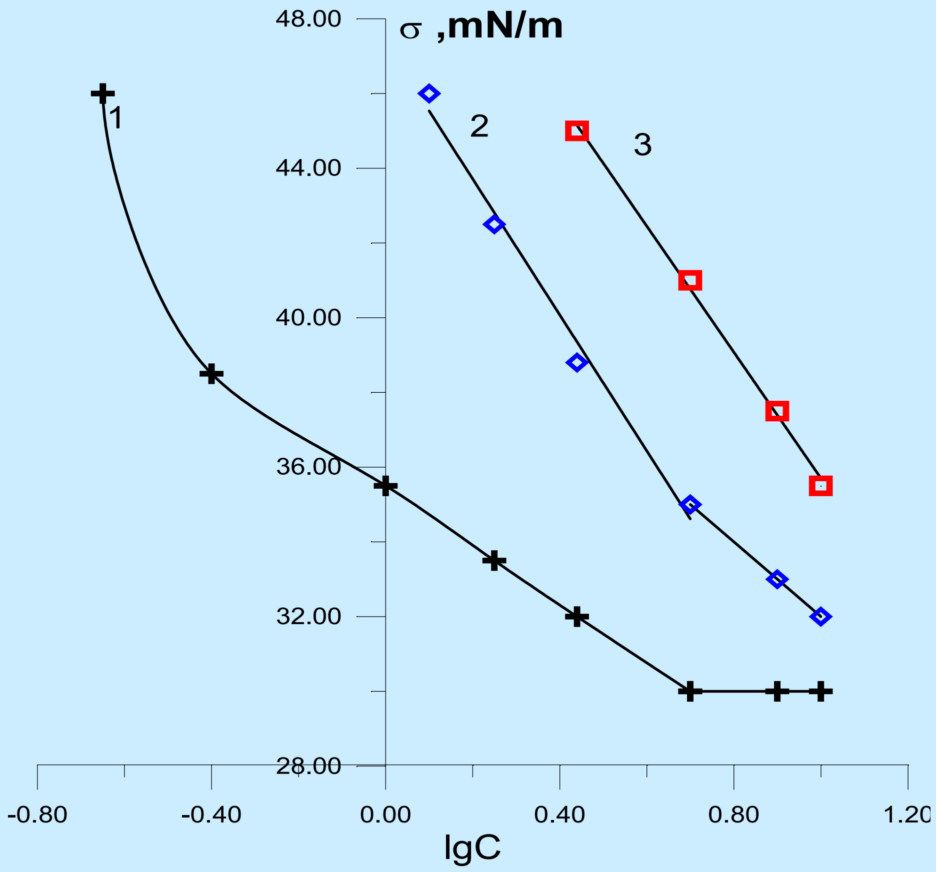
General scheme of tailored synthesis of functional oligomers with side ditertiary peroxide fragments



Colloidal-chemical characteristics of peroxide-containing functional oligoelectrolytes.



Isotherms of surface tension of FOS NVP-VEP-MA-VA 30:10:20:40 at: 1 – pH=3.2; 2 – pH = 6.96; 3 – pH=10.5

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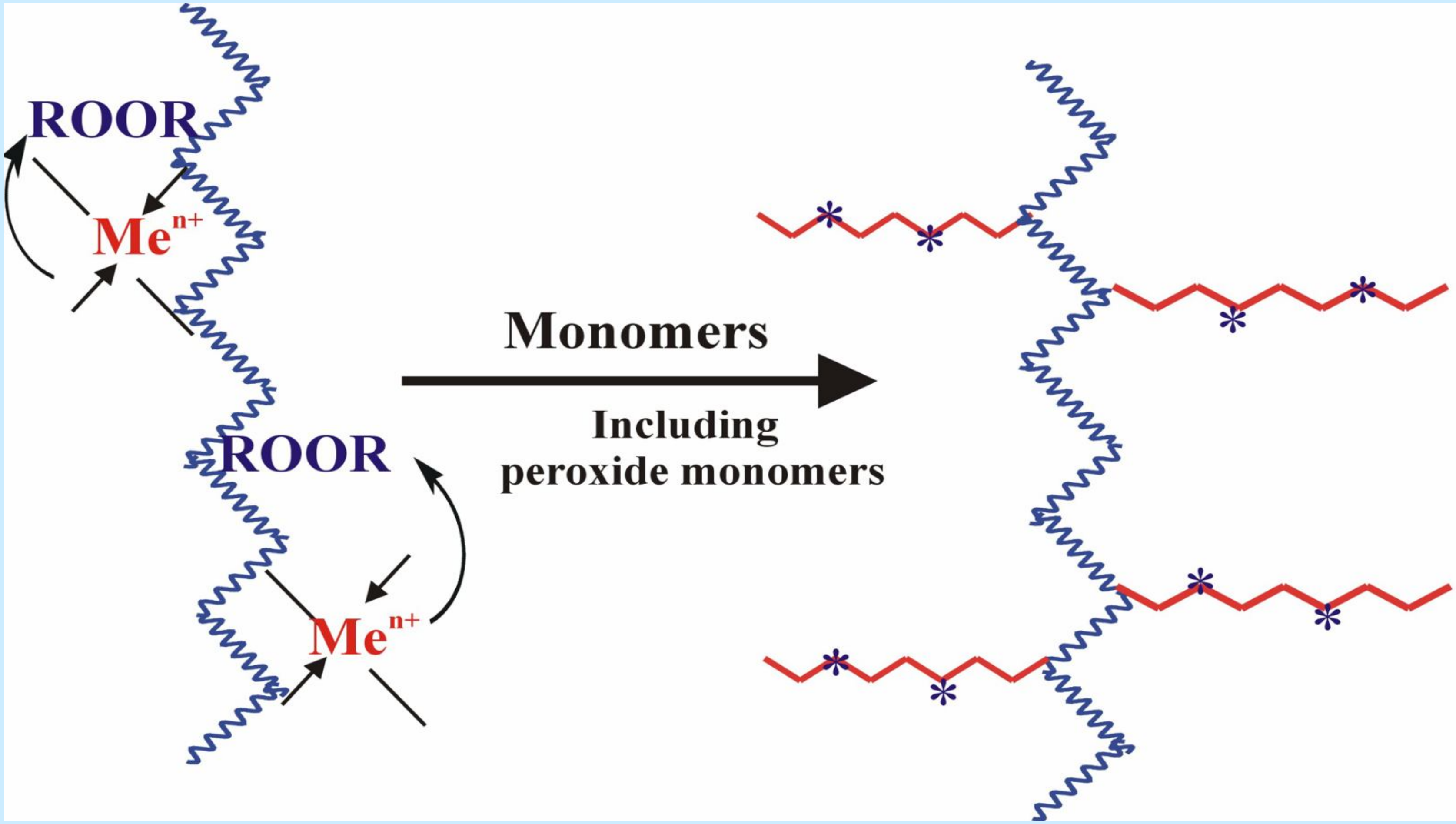
General scheme of tailored synthesis of functional oligoelectrolyte surfactants of comb-like structures



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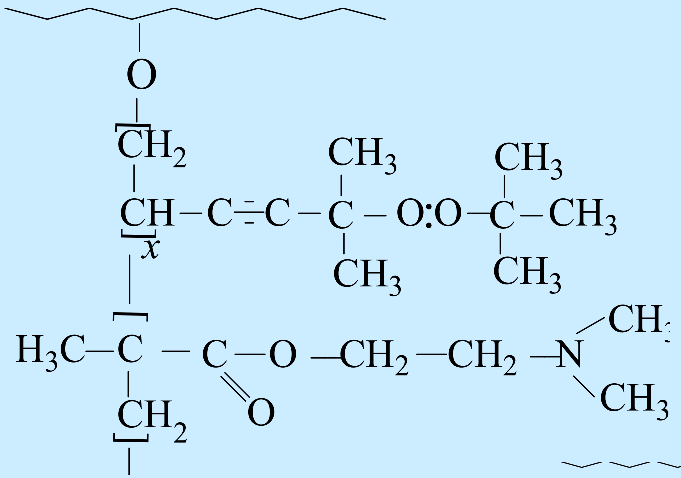
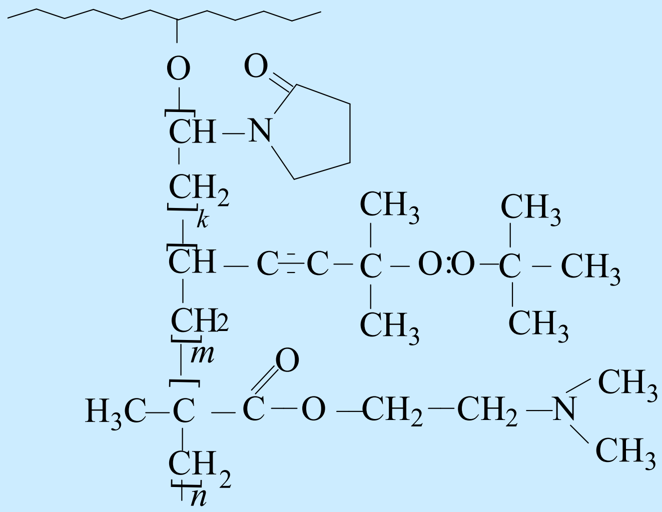
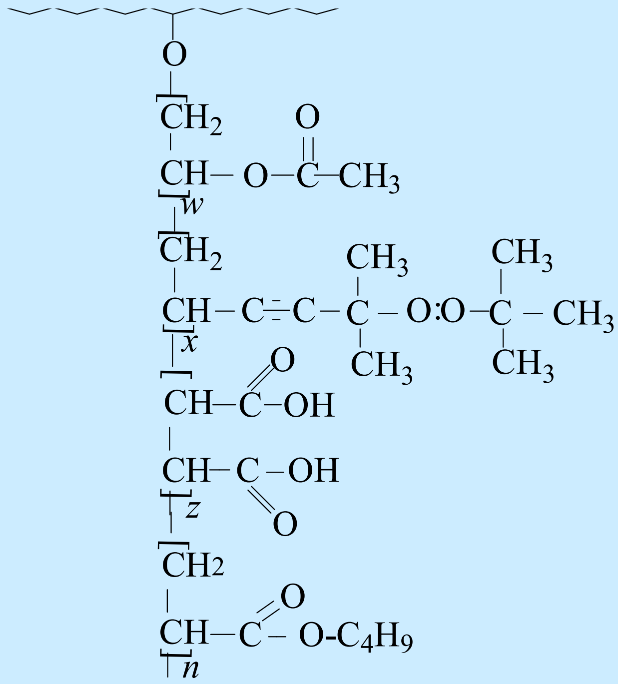
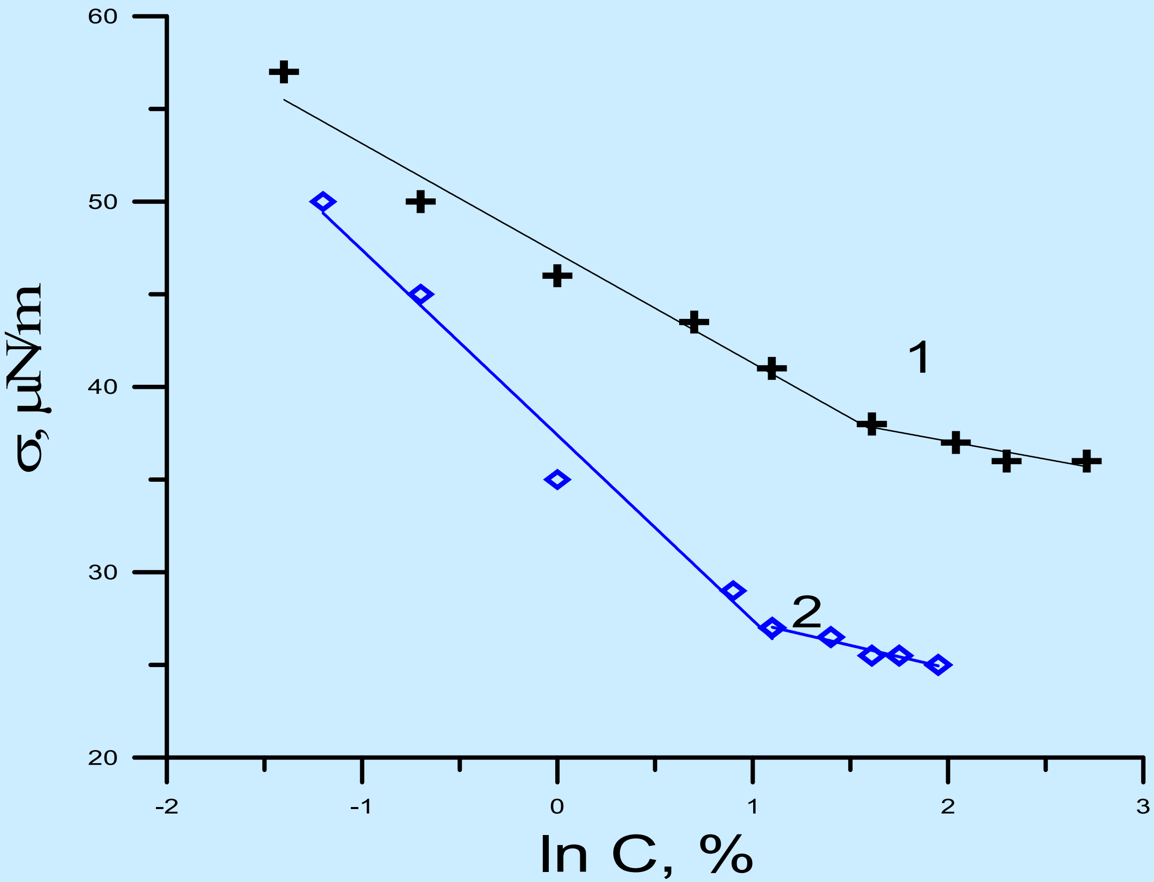
Peroxide containing comb-like oligoelectrolytes







Colloidal-chemical characteristics of peroxide-containing functional oligoelectrolytes of comb-like structure.



Surface tension isotherms of OMC-precursor (1) and comb-like OMC-graft-poly St (2) on its basis in water-ammonium solution

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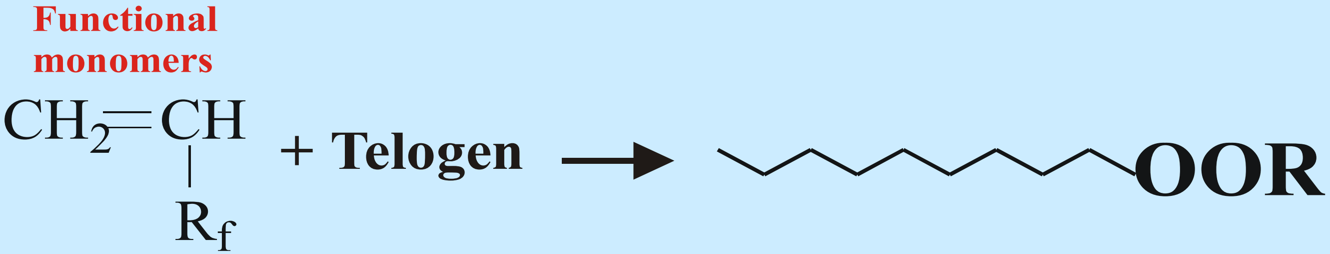
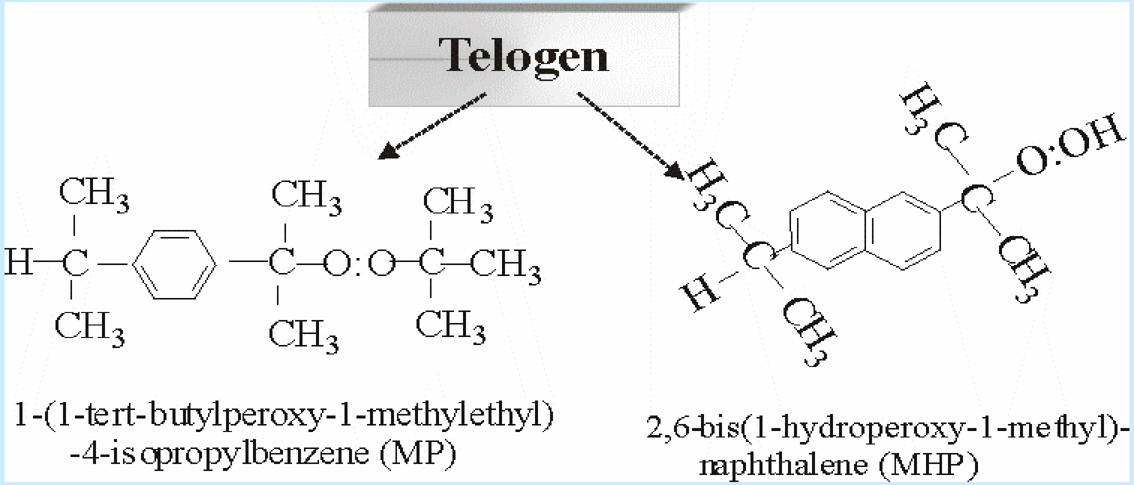
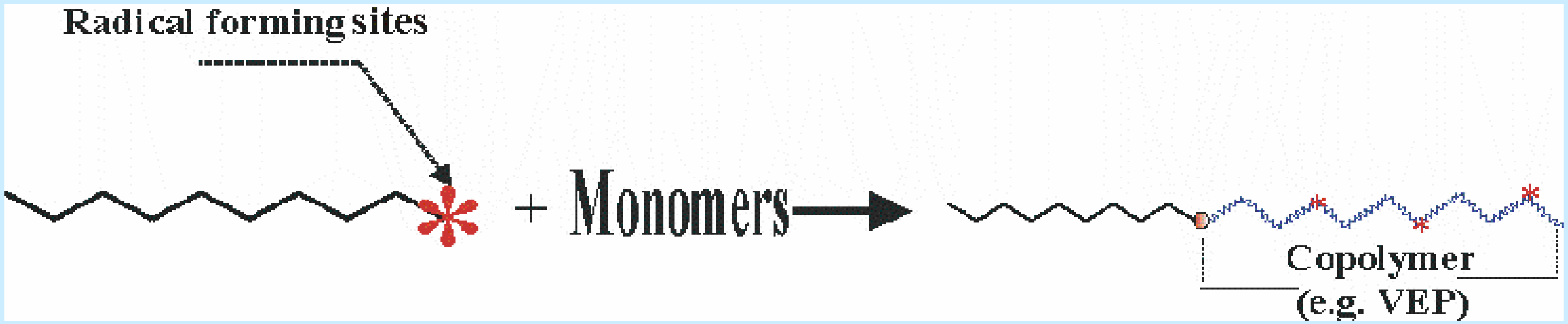
General scheme of tailored synthesis of telechelic oligoperoxides and derived oligoelectrolytes of block structure







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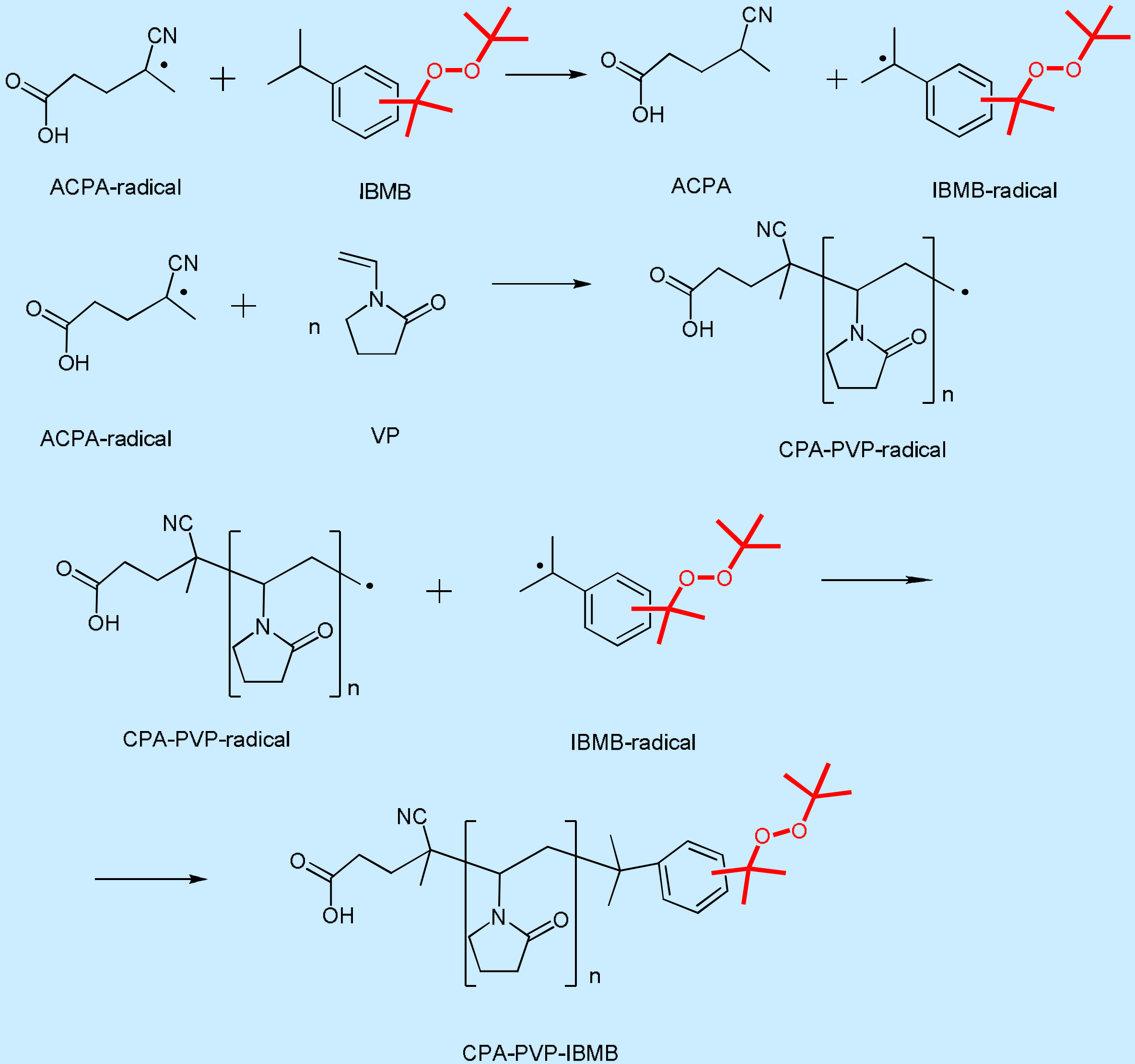
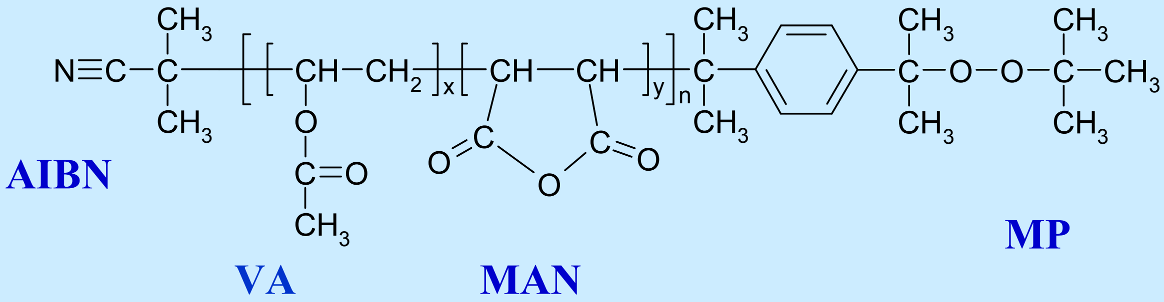
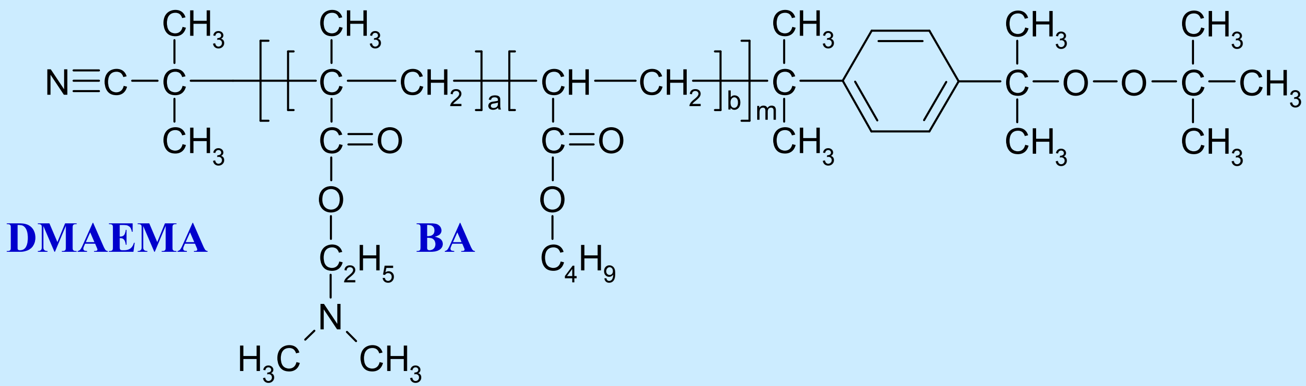
The scheme of the formation of heterotelechelic oligoperoxide surfactant



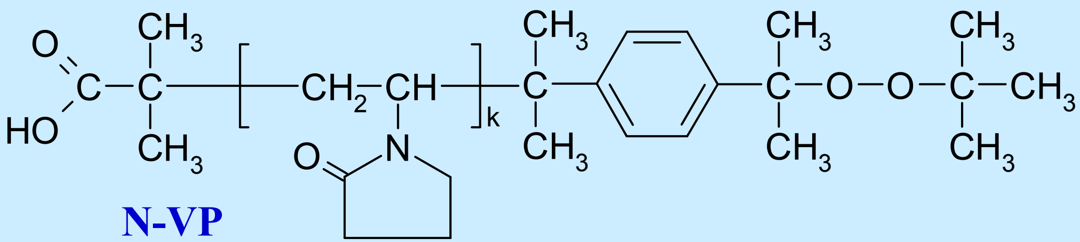
General structures of telechelic oligoperoxide surfactants



oligo(VA-MAN)-MP



oligo(DMAEMA-BA)-MP



oligo(N-VP)-MP

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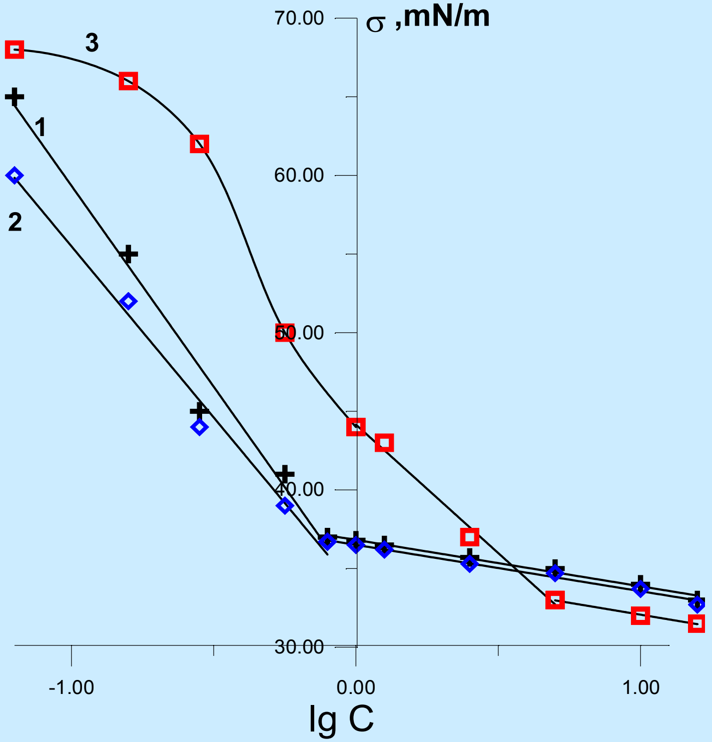
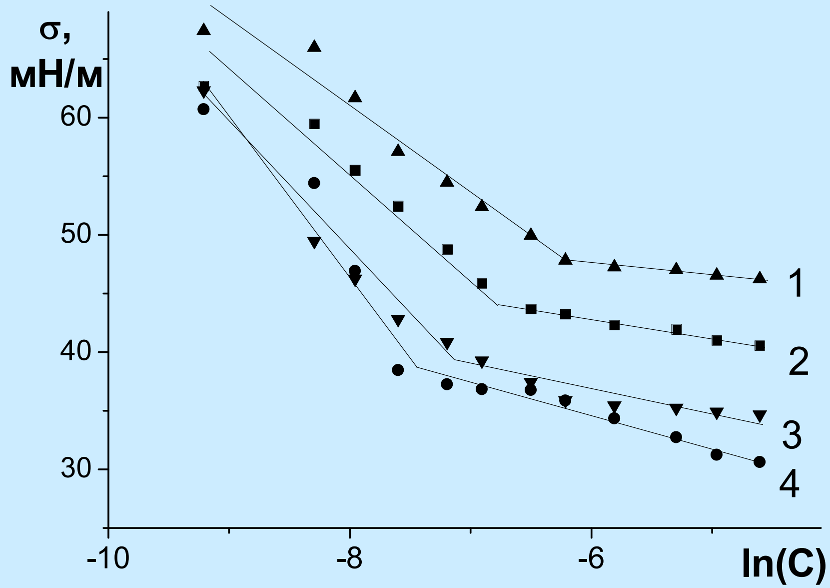
The dependences of surface activity of oligoelectrolyte water solutions on chain nature and pH value

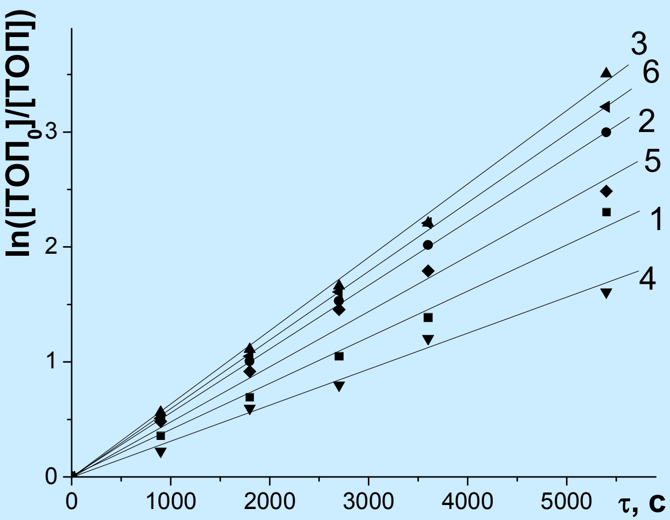


Isotherms of surface tension: 1 – FOS VAL-MP-MA 66.7:4.8:28.5 modified by PEG-13 at pH=7; 2- FOS VAL-MP-MA 66.7:4.8:28.5 modified by PEG-13 at pH=10; 3- FOS VAL-MP-MA 66.7:4.8:28.5 at pH=10



Isotherms of surface tension of oligoelectrolyte water solutions: oligo(VA-MAN)-MP (1, 3) and oligo(DMAEMA-BA)-MP (2, 4). pH: 2 (4); 7 (1,3); 12 (2).

Characteristics of thermal decomposition of MP and MP fragments in oligoperoxides

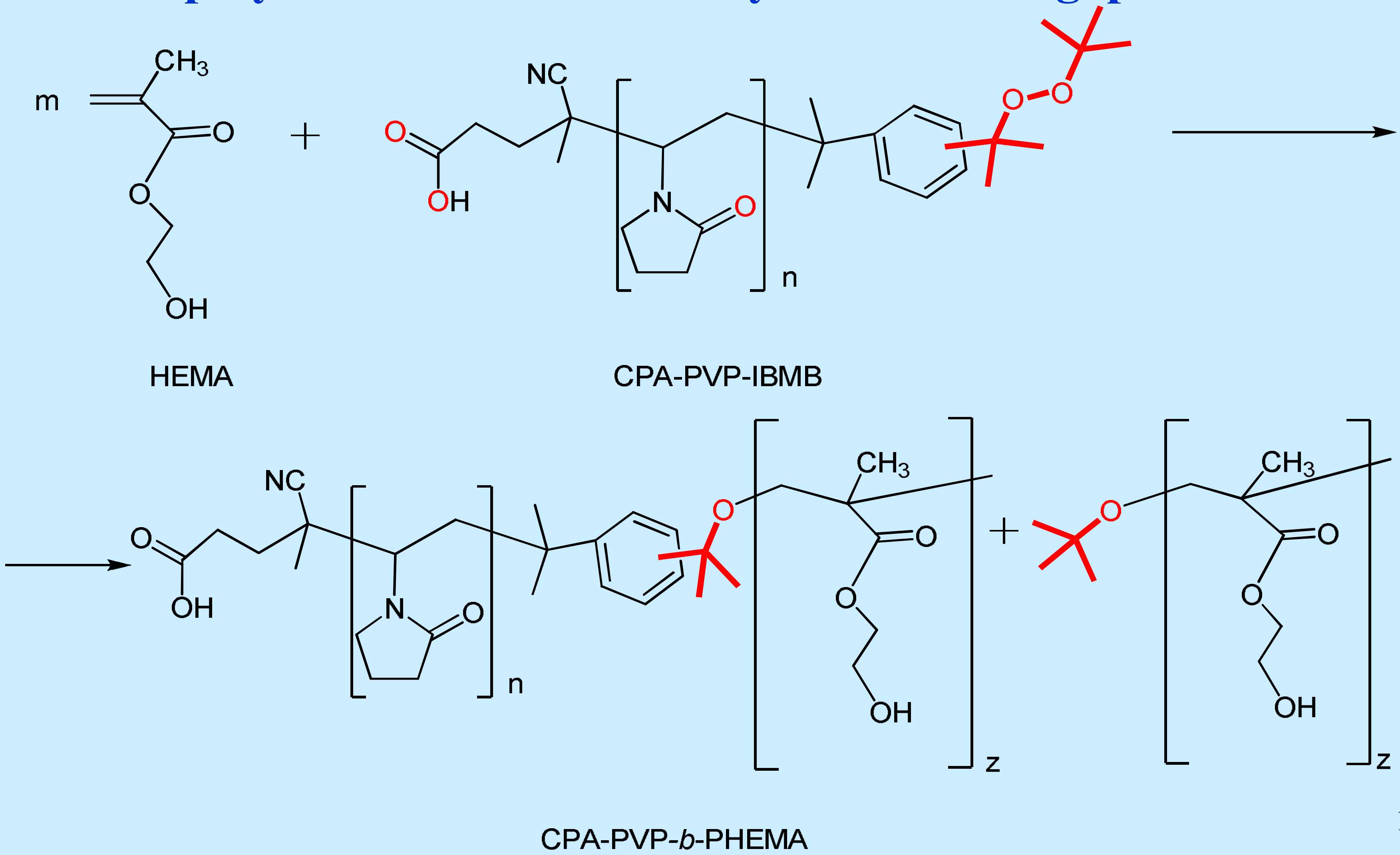


Semi logarithmic anamorphoses of kinetic curves of oligoperoxide decomposition in water - T=403K, $[MP]_0$, mmole/l: oligo(VA-MAN)-MP: 1 – 0.4; 2 – 1.2; 3 – 2.0; oligo(DMAEMA-BA-MP): 4 – 0.4; 5 – 1.2, 6 – 2.0.

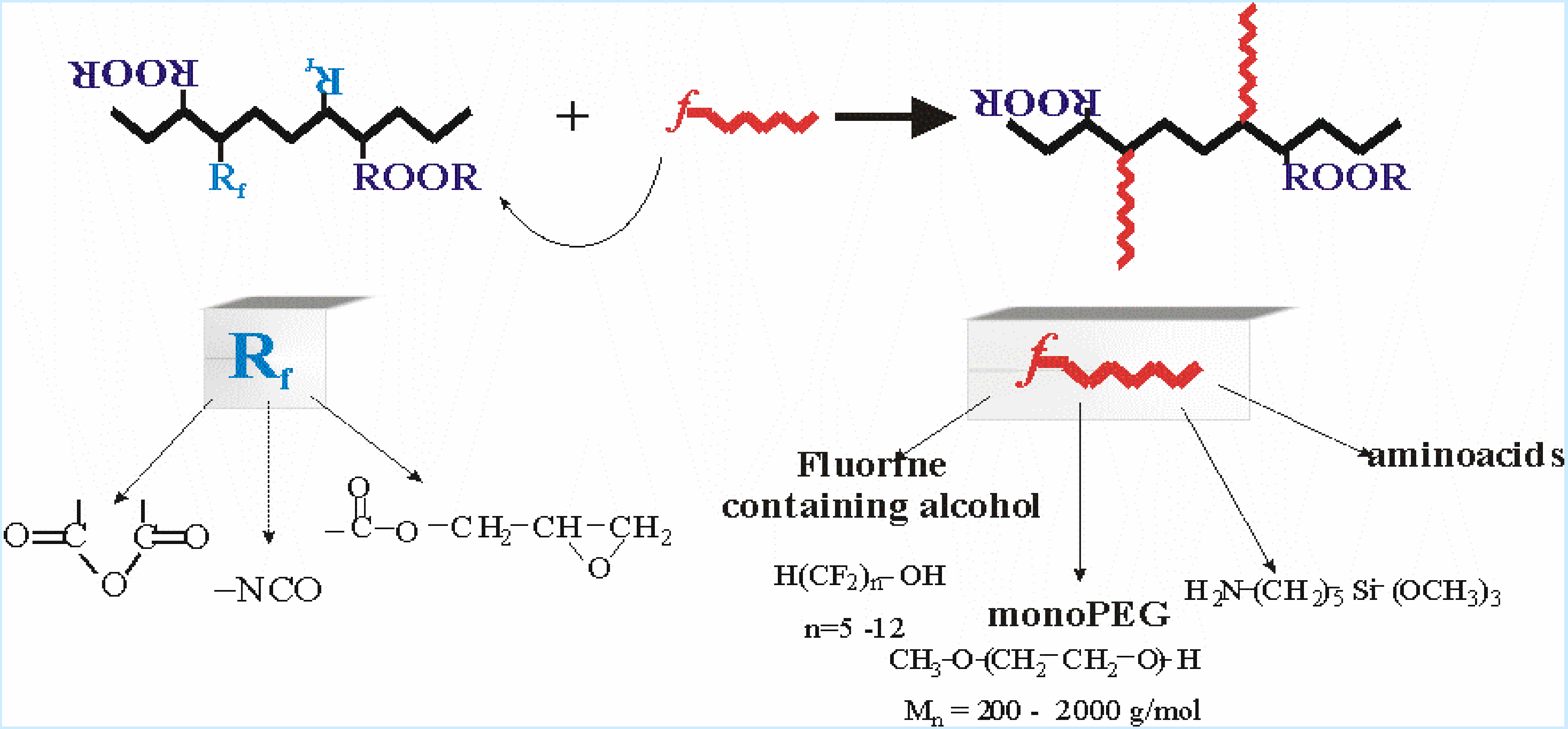
Activation energy and entropy changes of the decomposition of MP and MP fragments in oligoperoxides

Substance	E_a, kJ/mole	$\Delta S^{\neq}$, J/(mole·K)
MP	167±5	15.6±1.3
Oligoperoxide in 1,4 - dioxane	132±7	0.7±0.1
Oligoperoxide in water	110±7	-12.4±1.2

The scheme of the formation of block-copolymer via radical polymerization initiated by telechelic oligoperoxide



Polymer analogous transformation of functional peroxide-containing surface-active oligoelectrolytes



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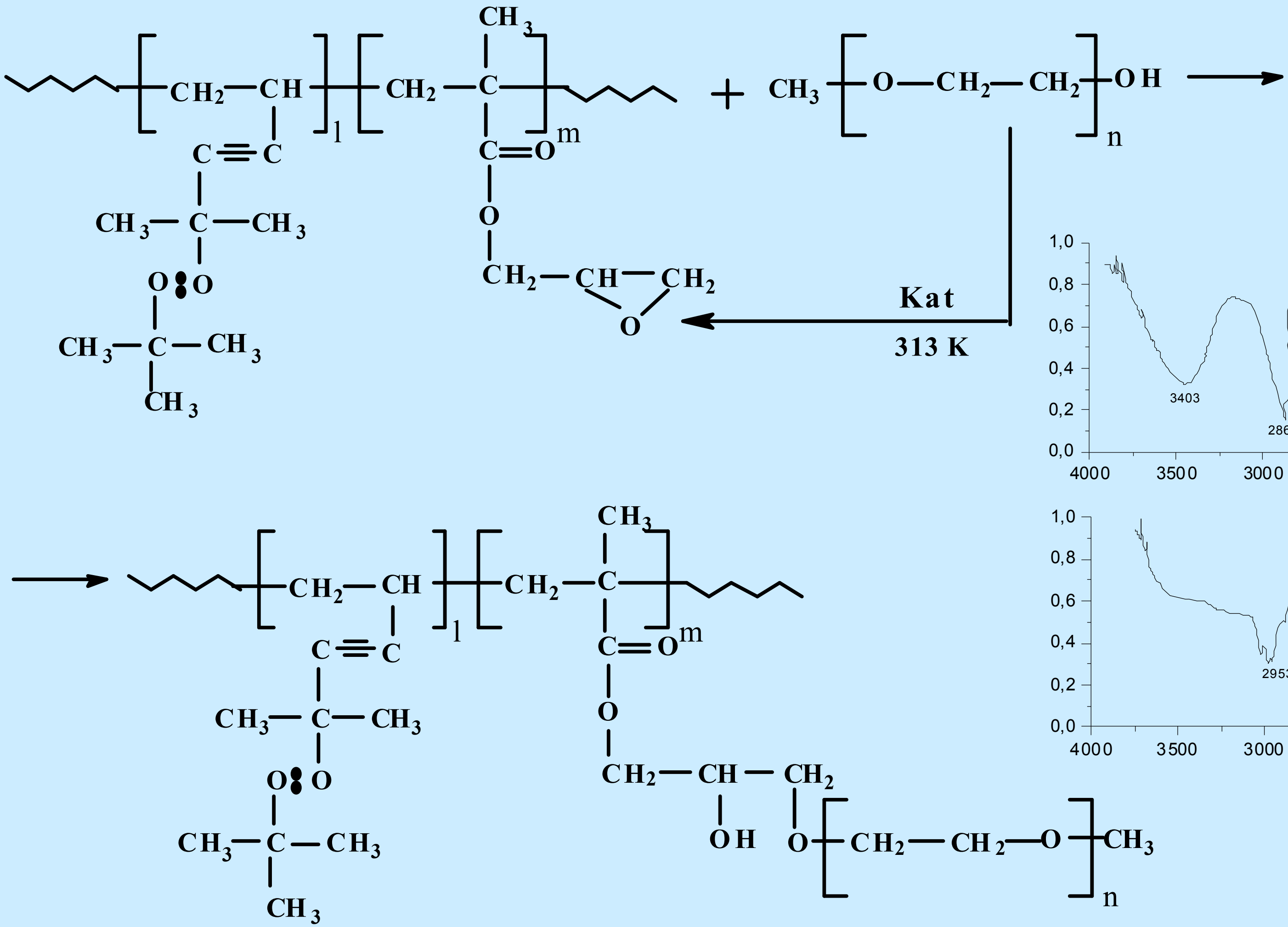
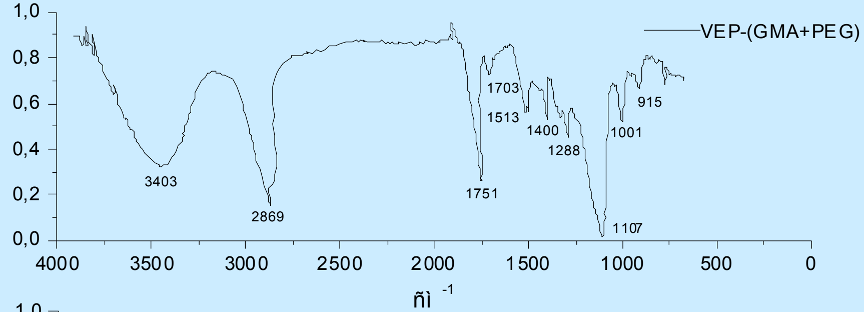
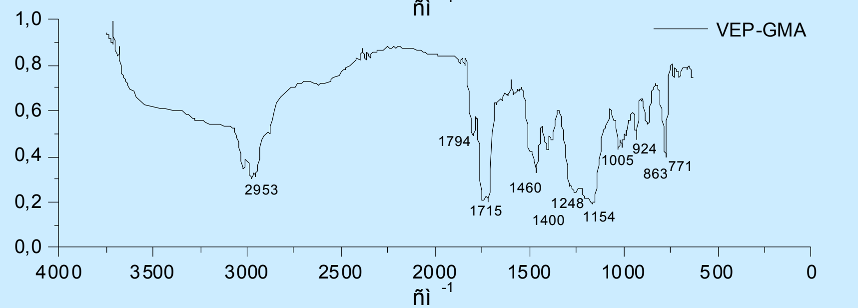
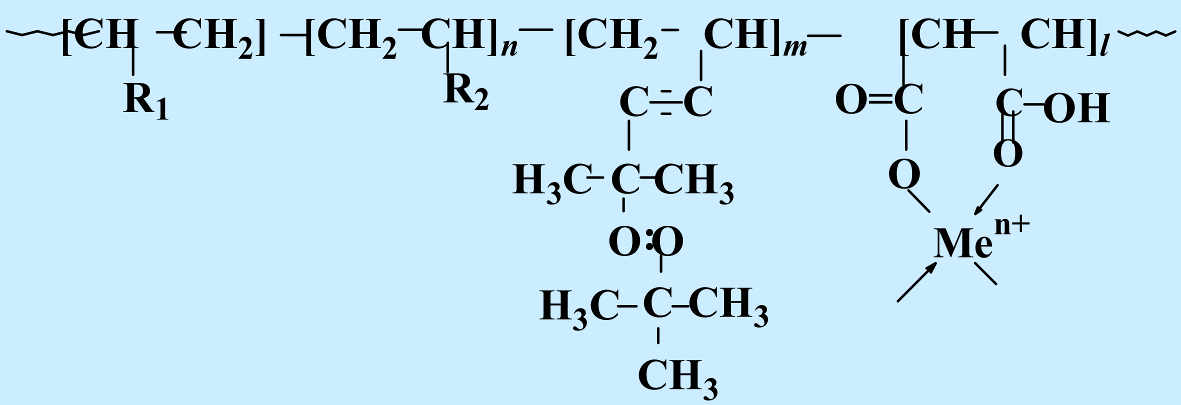
The general scheme of oligoperoxide surfactant PEGylation and comparative spectra of initial and PEGylated oligoperoxide

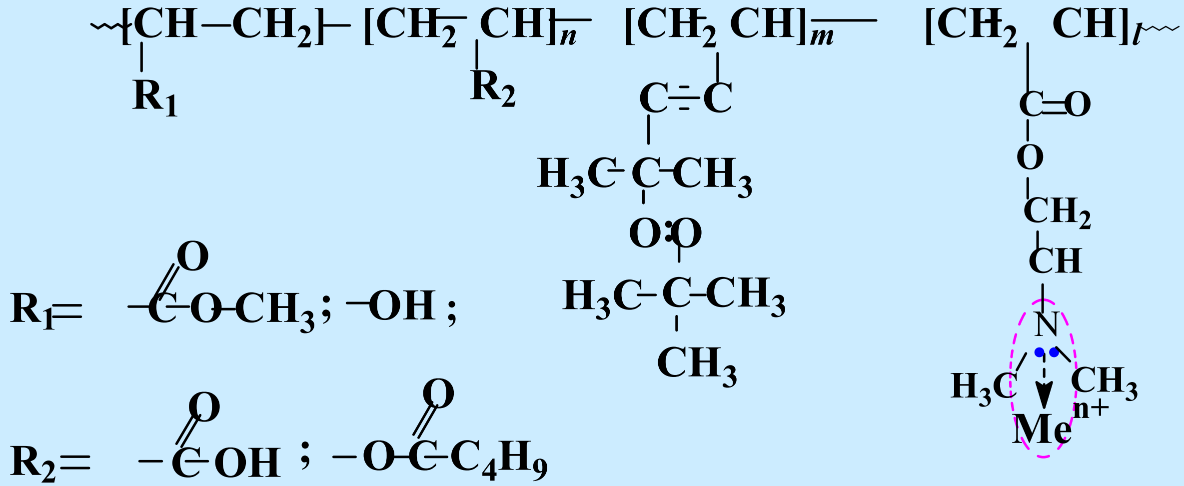






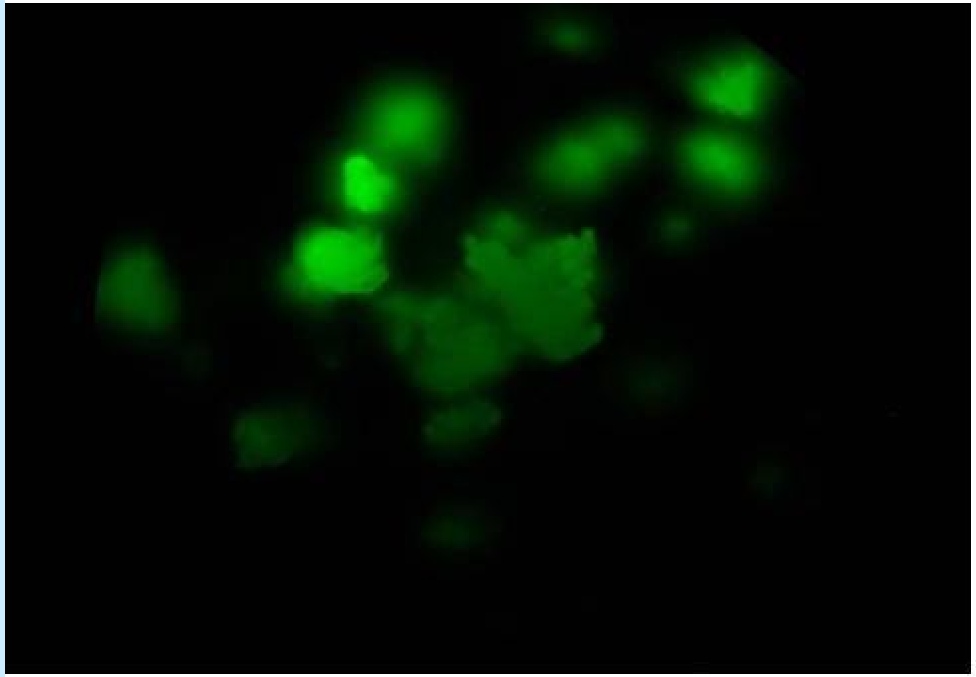
Coordinating metal complexes of transition and of rare earth metal cations



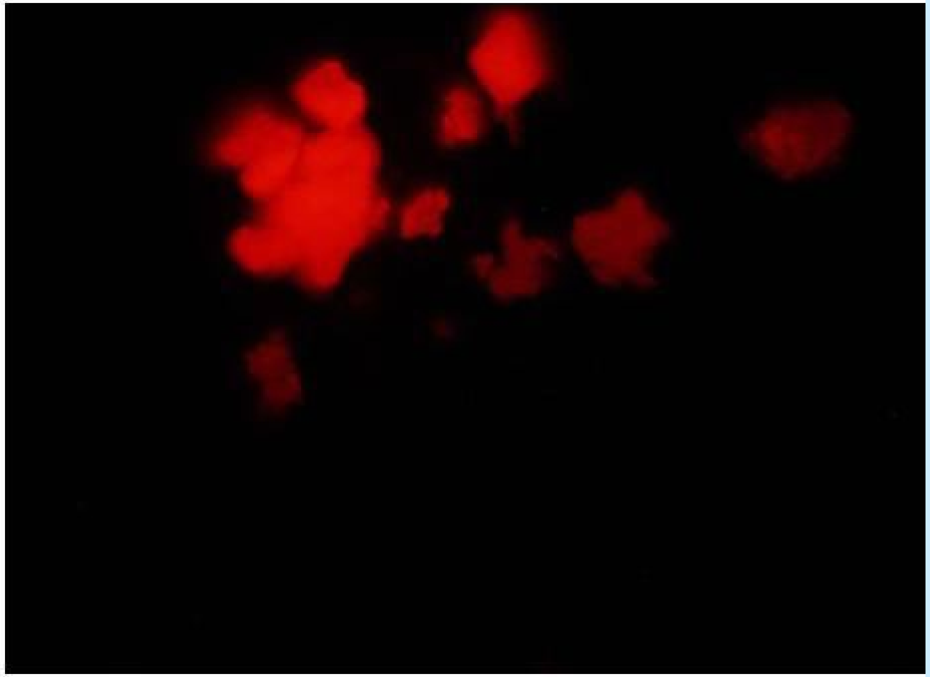


$Me^{n+} = Cu^{2+}, Co^{2+}, Ni^{2+}, Cr^{3+}, Eu^{3+}, Ce^{3+}$

Luminescence spectrum of the oligoelectrolyte surfactant and derived Eu^{+3} complex



Luminescence of surface-active oligoelectrolyte surfactants



Luminescence of surface-active coordinated oligoperoxide complex of Eu^{+3}

Why these oligoperoxide based oligoelectrolytes?

•controlled design of a structure

• controlled molecular weight (1,000 – 30,000g/mole)
•narrowed molecular weight distribution

• controlled macro and microstructure
• controlled functionality and reactivity

• controlled solubility, surface activity
•biocompatibility and non toxicity

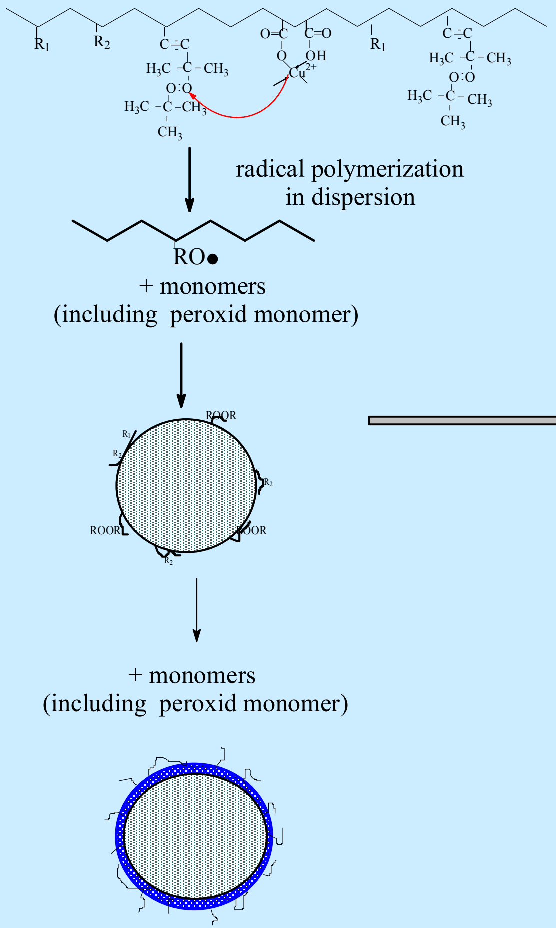
•Capability to form free radicals and initiate radical reactions

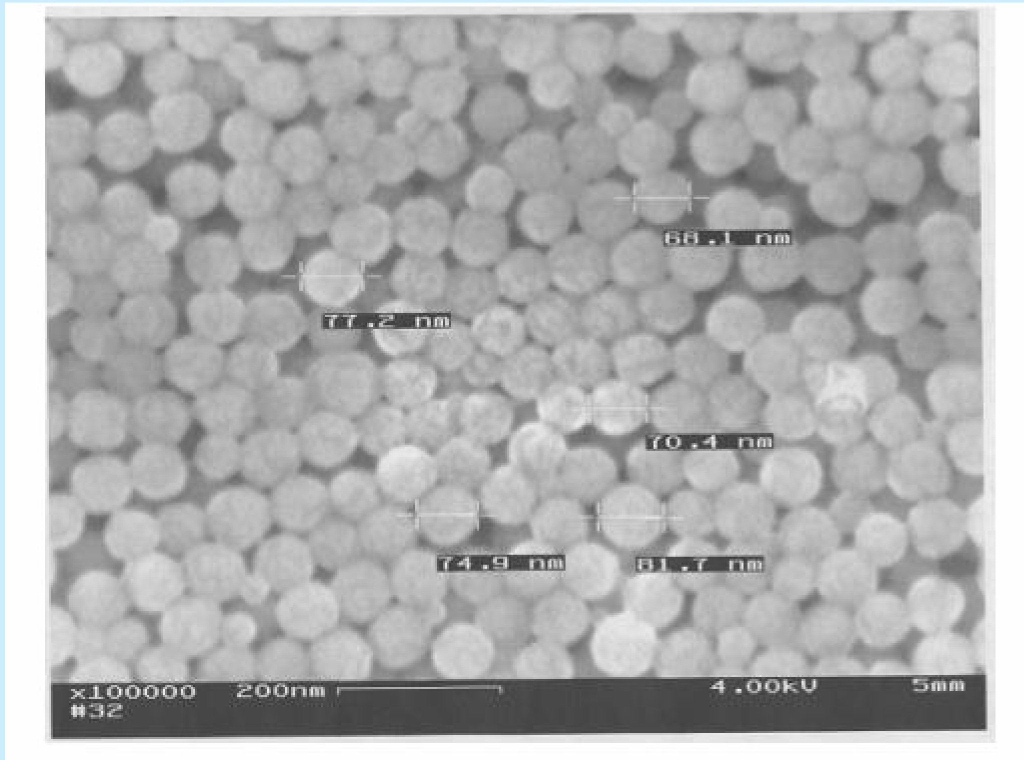
2. Luminescent, coloured and magnetic nanocomposites for pathological cell detection, tagging and treatment (main approaches for the synthesis and functionalization).

Main approaches of the synthesis and functionalization:

1. Dispersion polymerization initiated with oligoperoxide surfactants including luminescent oligoperoxide metal complexes as initiators and stabilizers;
2. Dispersion polymerization with unsaturated surfactants and dyes;
3. Micro encapsulation with participation of oligoperoxide surfactants as surface modifiers and initiators;
4. Homogeneous nucleation of metal and oxide nanoparticles and colloids in the presence of oligoperoxide surfactants as templates and surface modifiers;
5. Sorption modification of polymer and mineral nanoparticles and seeded polymerization initiated from the surface

Water dispersion polymerization of styrene, butyl methacrylate, methyl methacrylate and monomer mixture initiated by oligoperoxide based coordinating metal complexes





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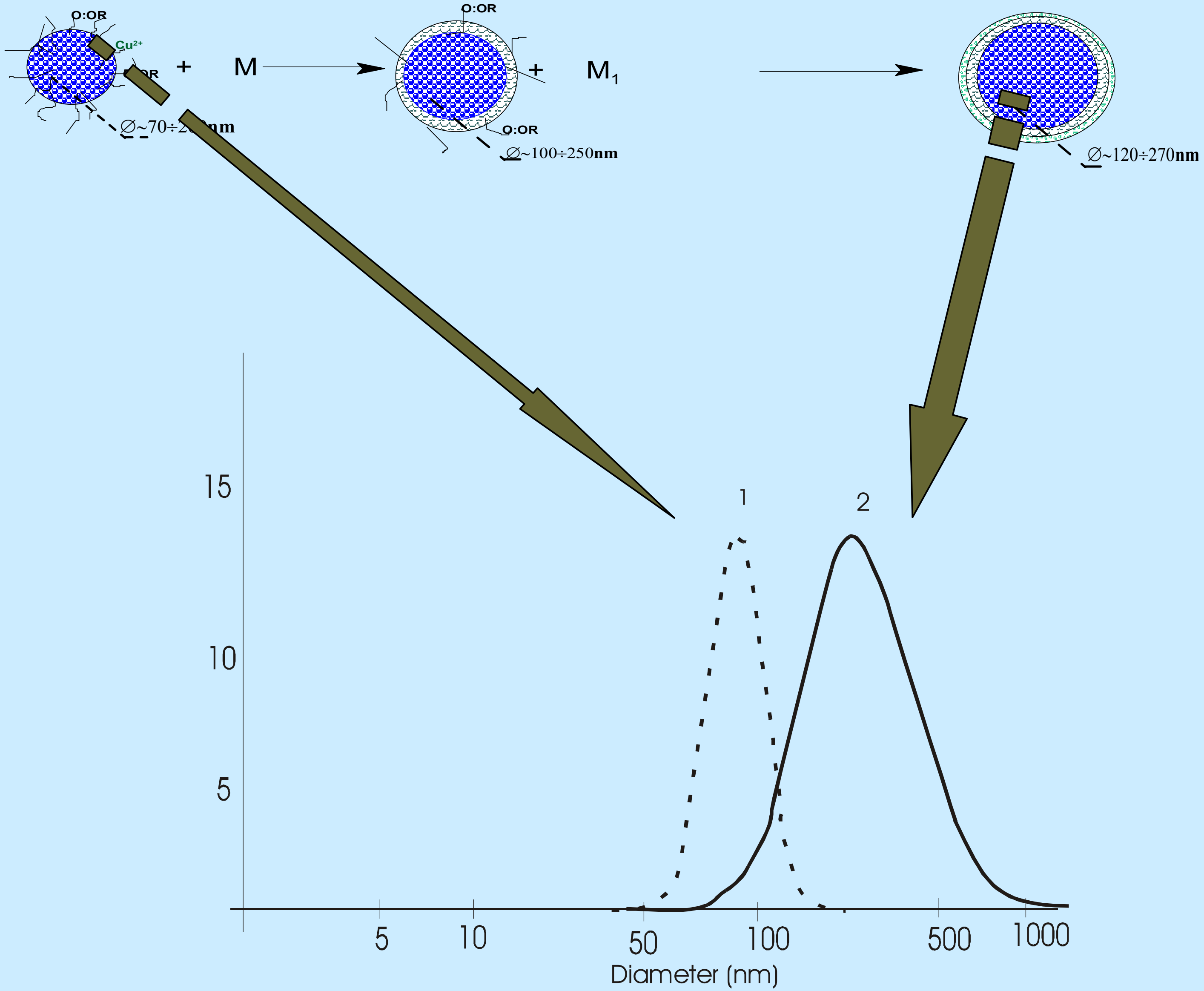
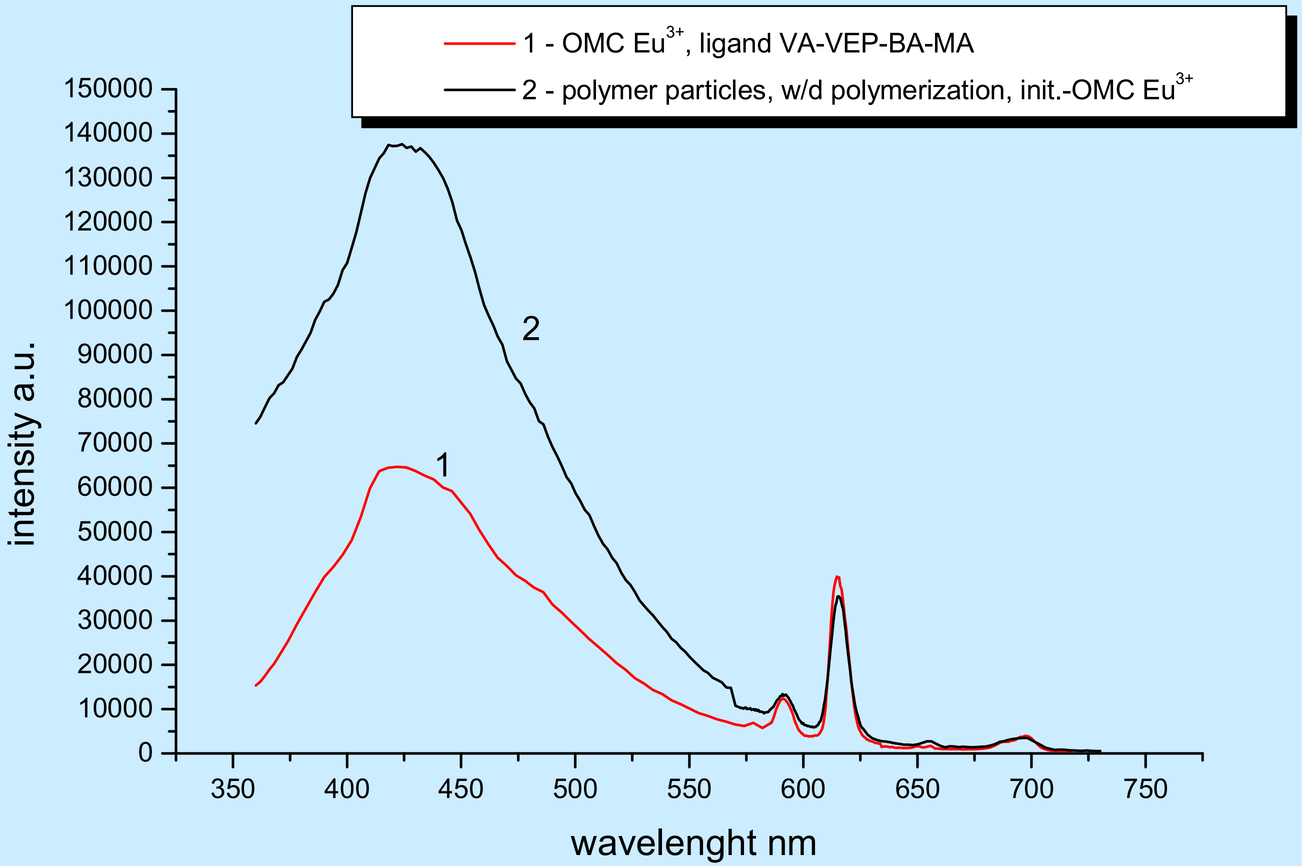
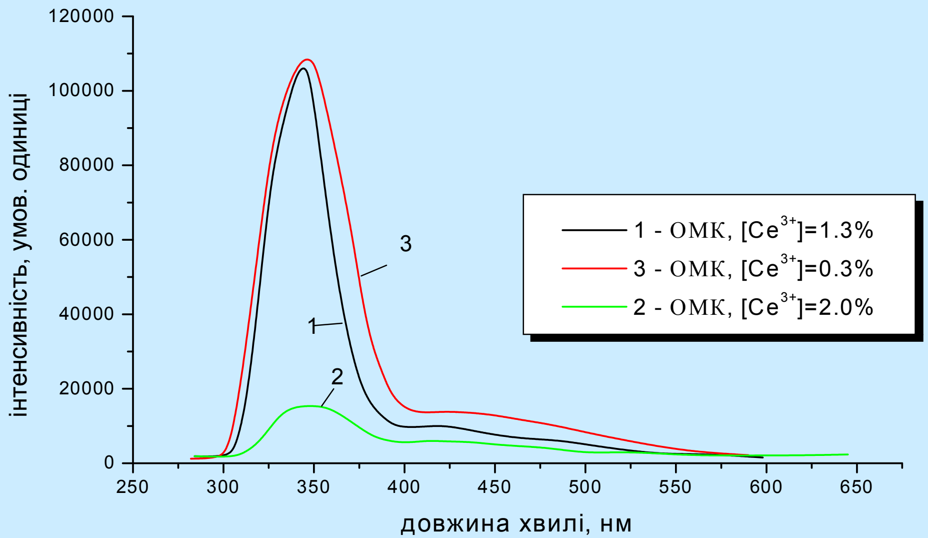
Seeded water dispersion polymerization initiated by oligoperoxide metal complexes immobilized on the primary particle surface

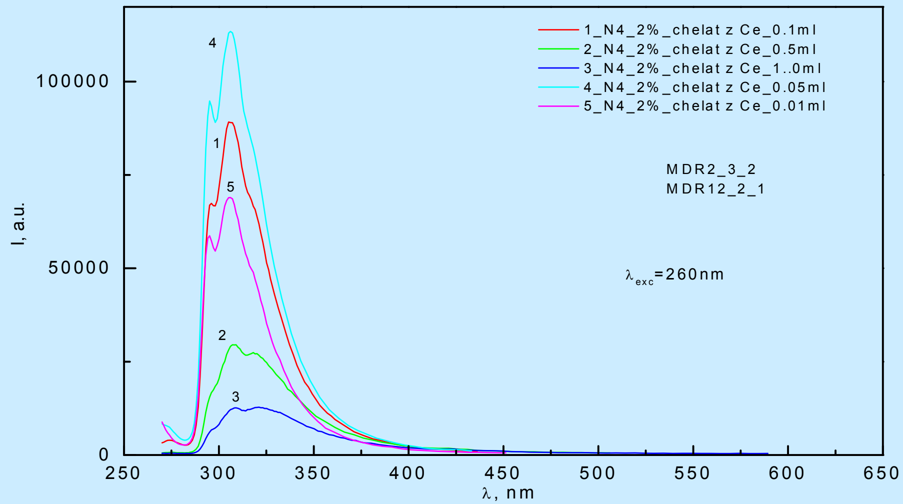


Luminescence spectrum of the complex and polymeric nanoparticles synthesized in the presence of coordinated oligoperoxide complex of Eu^{+3} as initiator and surface modifier



Synthesis of luminescent functional polymeric nanoparticles

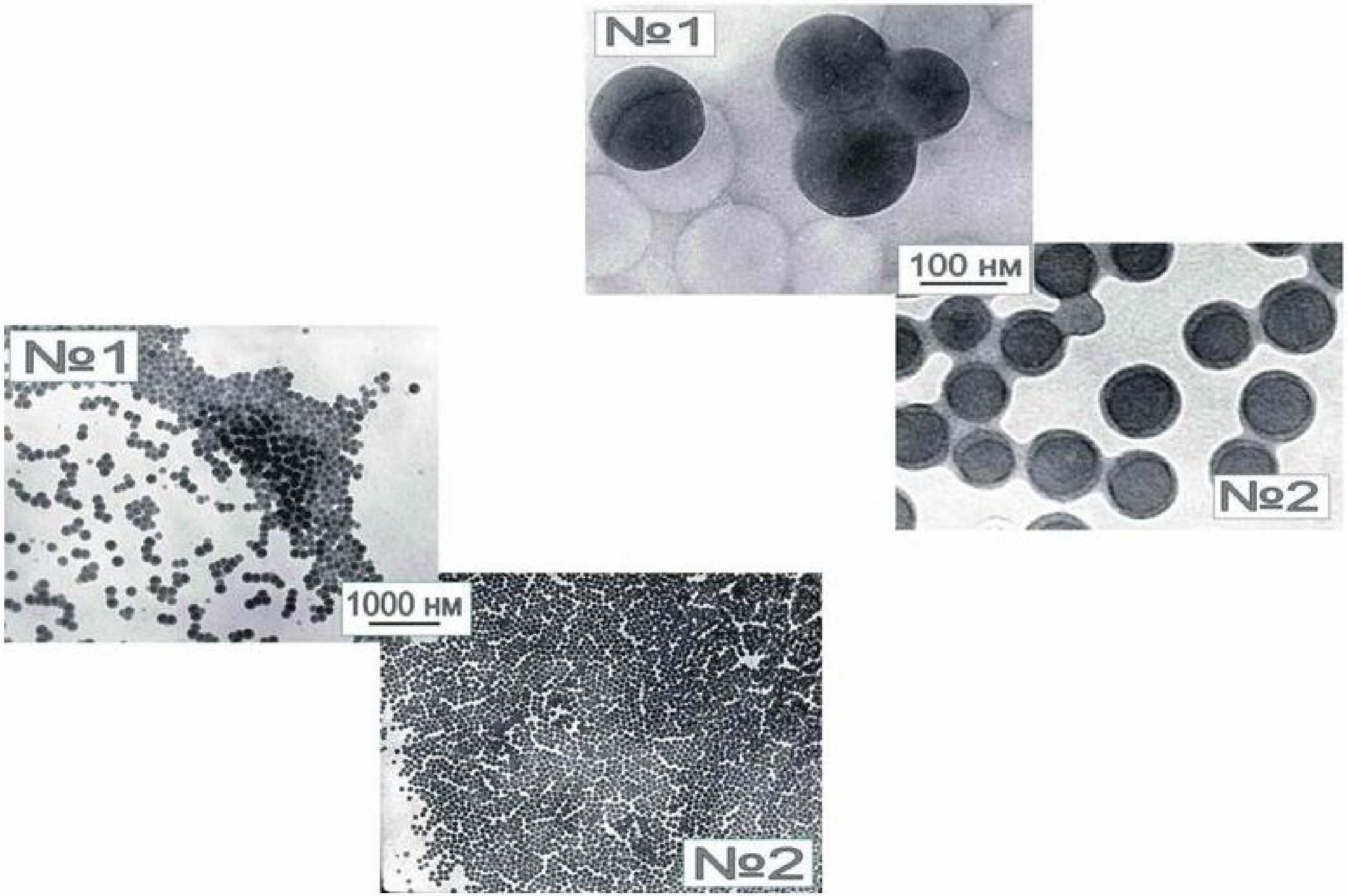




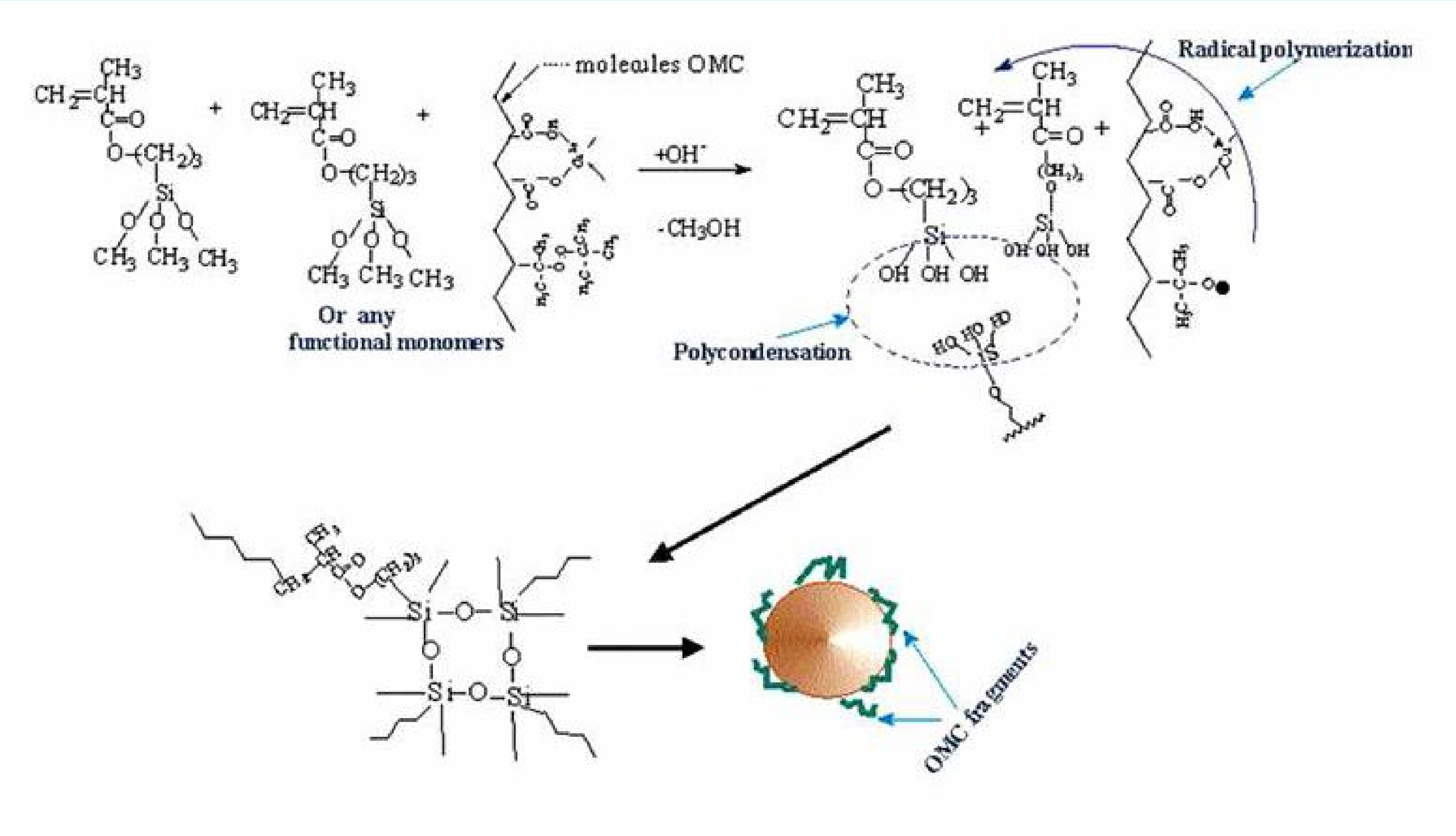
Spectra of luminescence intensity distribution in oligoperoxide metal complexes [Ce^{3+}]: 1 - 1.3%; 2 – 0.3%; 3 - 2,0 % per oligoperoxide.

Spectra of luminescence of polymeric particle in water systems based on Ce^{+3} coordinated oligoperoxide VA-VEP-BA-MA complexes with [Ce^{3+}] =0.09% (1, 2, 3), [Ce^{3+}] =1.27% (4, 5) and Ce^{+3} coordinating oligoperoxide VA-VEP-BA-MA-unsaturated dye complexes (6, 8, 10). Complex concentration in water: 1, 6, 8, 10 = 3%; 2 = 2%; 3, 5 = 1%; 4 = 5%

TEM picture of polystyrene nanoparticles with functional shell containing coordinated Ce^{+3} cations (samples 1 and 2 are 2 and 3 % of Ce oligoperoxide complex per H_2O ($[Ce^{3+}]$ =1.25%)



The scheme of formation of functional polymer-mineral nanoparticles consisting of cured SiO_2 core and oligoperoxide shell



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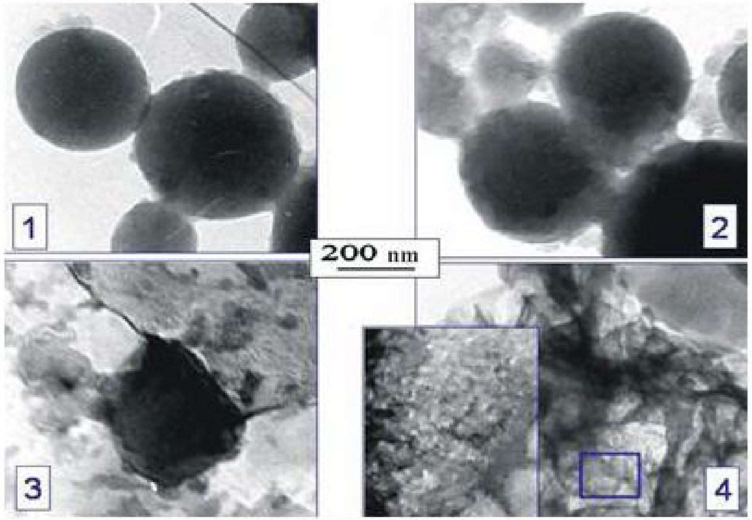
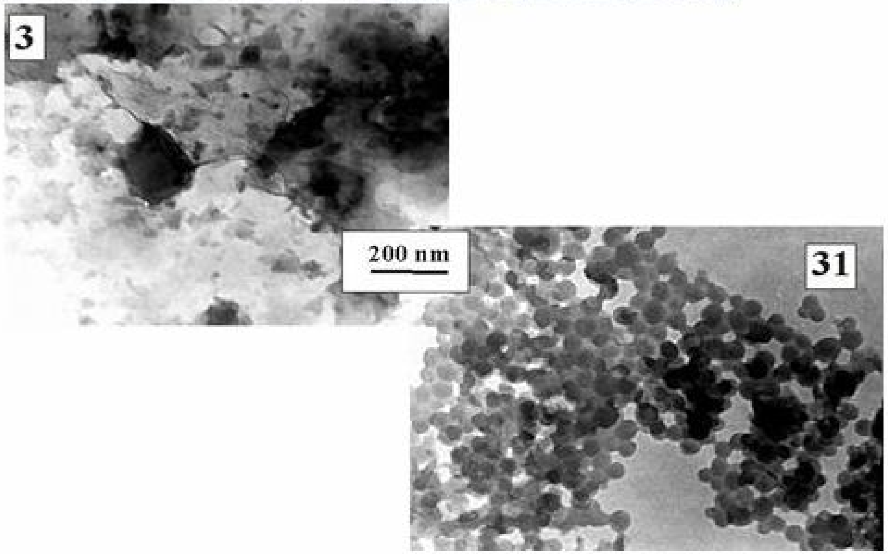
TEM images of functional polymer-mineral nanoparticles consisting of cured SiO_2 core and oligoperoxide shell

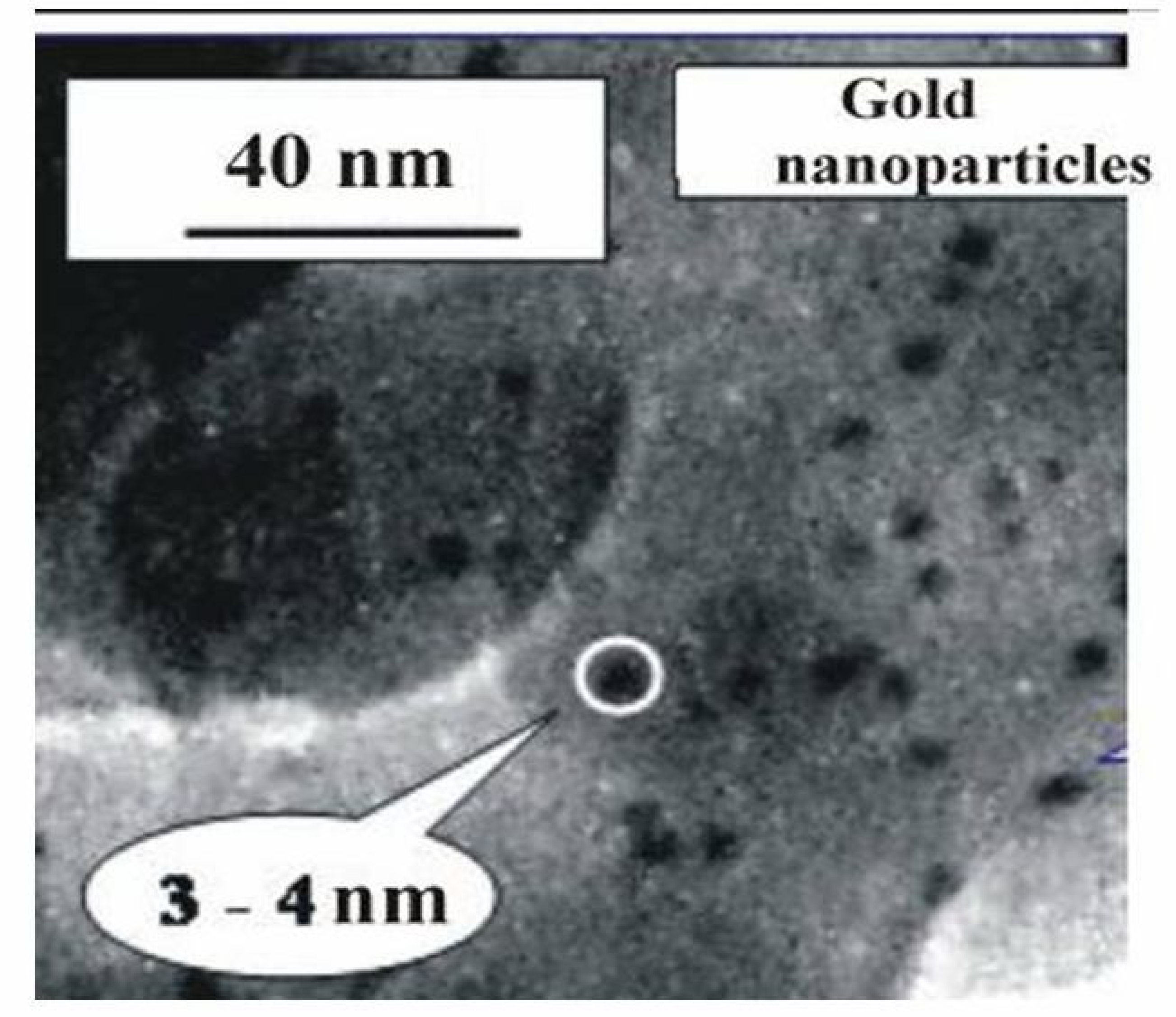


TEM images of functional SiO_2 nanoparticles synthesized via water dispersion polymerization of A174 initiated OMC at pH=12 (1, 2), 8.5 (3, 4) [OMK]= 0.5% (1,3) and 3% (2,4)



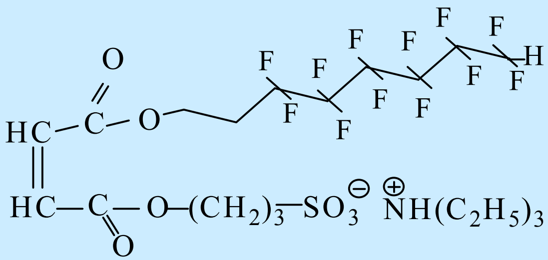
TEM images of functional SiO_2 nanoparticles synthesized via water dispersion polymerization of A174 initiated by OMC 0.5% (3) at pH= 8.5 SiO_2 nanoparticles synthesized via seeded polymerization initiated from the surface of nanoparticles #3

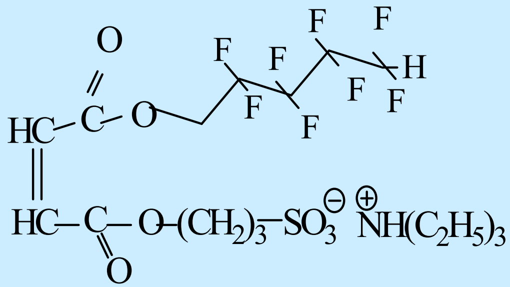
GOLD NANOPARTICLES IN FUNCTIONAL SHELL OF SiO_2 PARTICLES



Dispersion polymerization with unsaturated surfactants and dyes

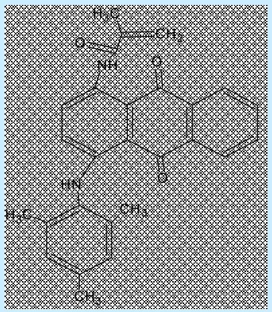
*S*urface-active monomers (SM) used for the co polymerization with Styrene



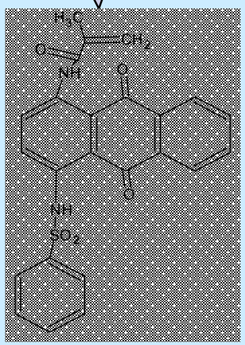


Unsaturated dyes used for the obtaining coloured functional polystyrene particles

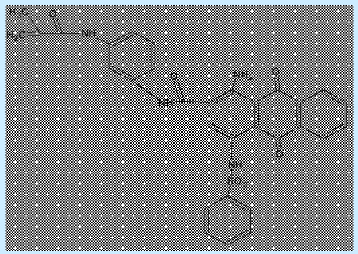
violet



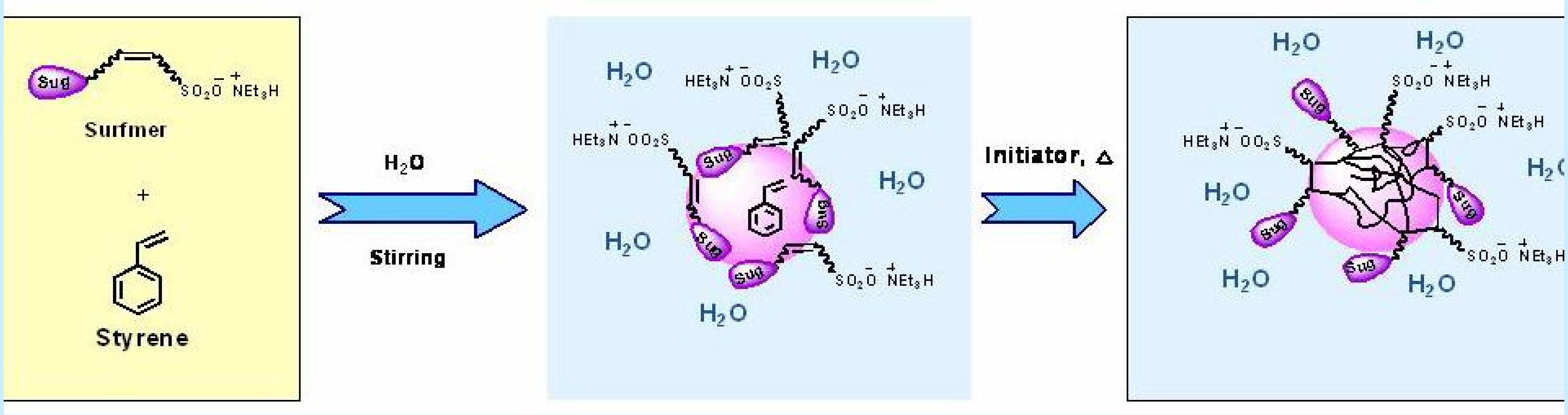
red

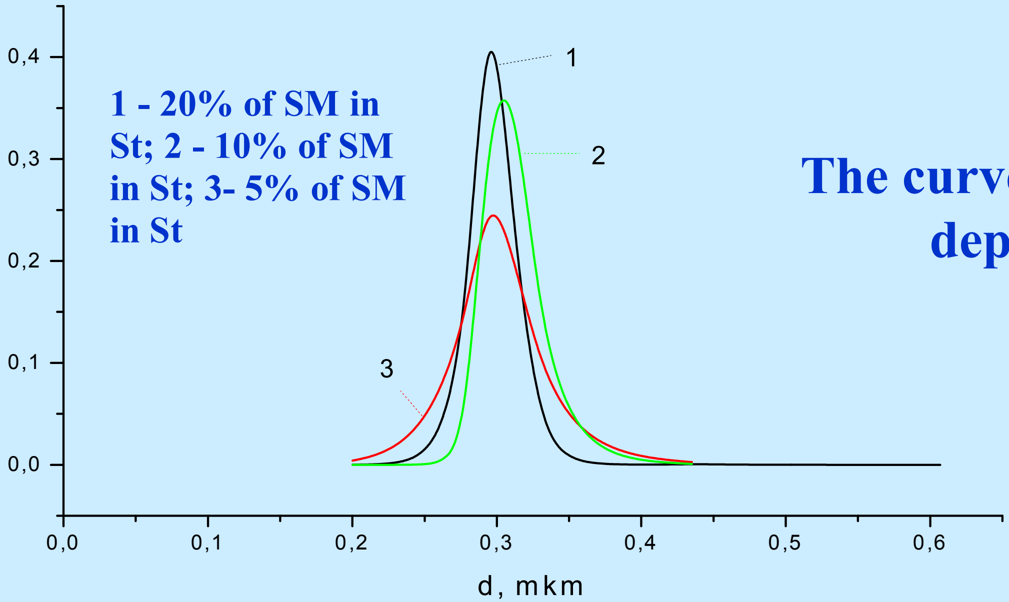


blue



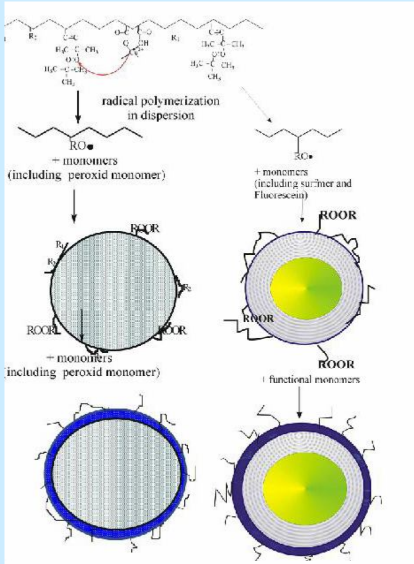
The scheme of water dispersion copolymerization of styrene with unsaturated surfactant

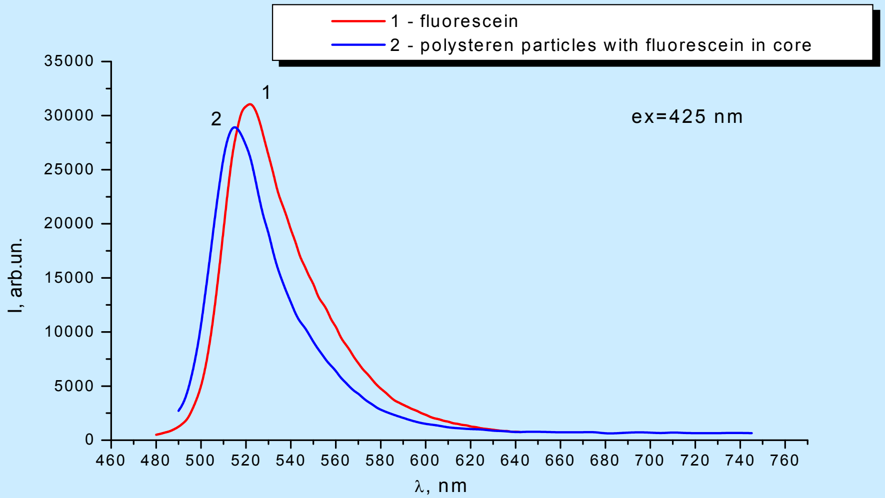




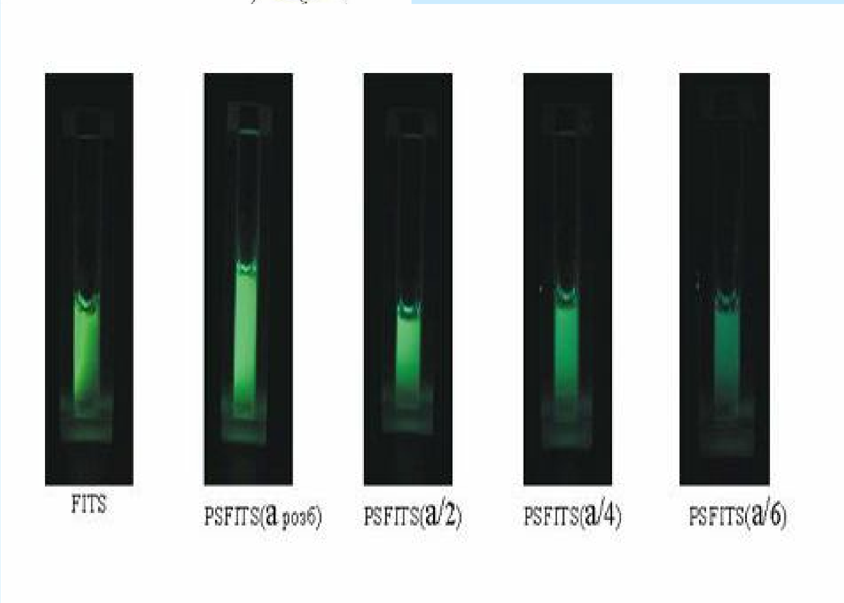
The curves of particle size distribution depending surfmer content

Micro encapsulation of fluorophores with participation of oligoperoxide surfactants





Luminescence spectra of fluorescein (1) and polystyrene particles with fluorescein in the “core”



Green fluorescence of FITS and FITC labeled polystyrene nanoparticles (PSFITS a) in water based systems at distinct dilution (PSFITS a/2, a/4, a/6)

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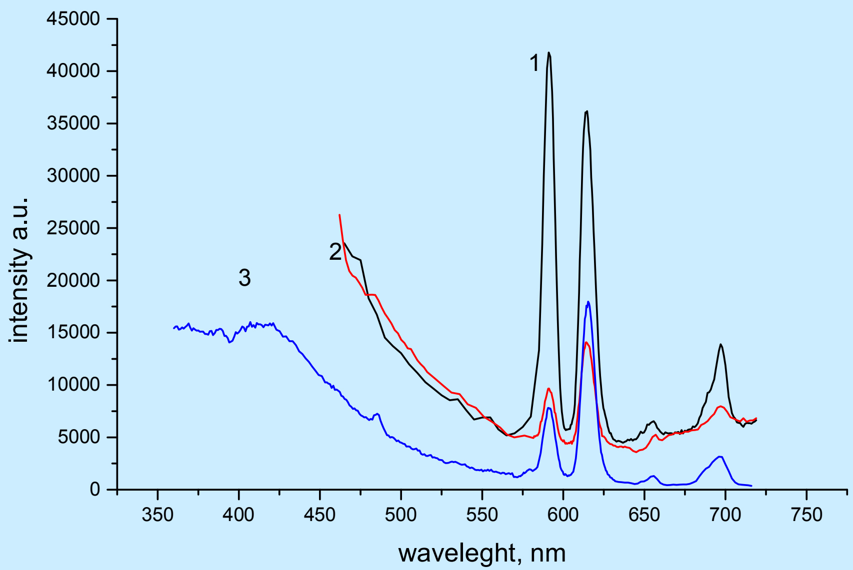
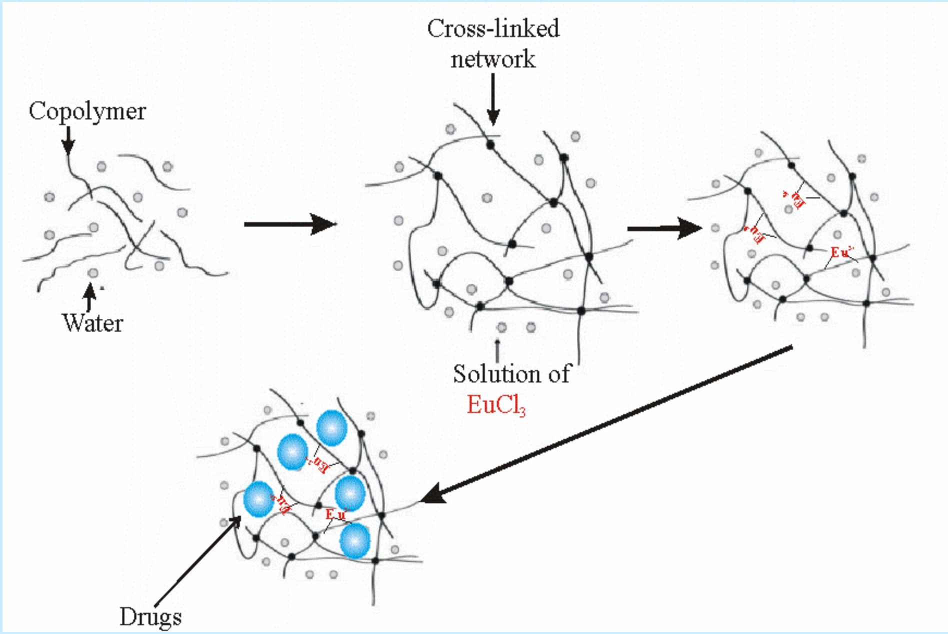
The synthesis of reactive functional oligoperoxide-containing cured nanogels with controlled porous size on the basis of copolymers of unsaturated acids or amines

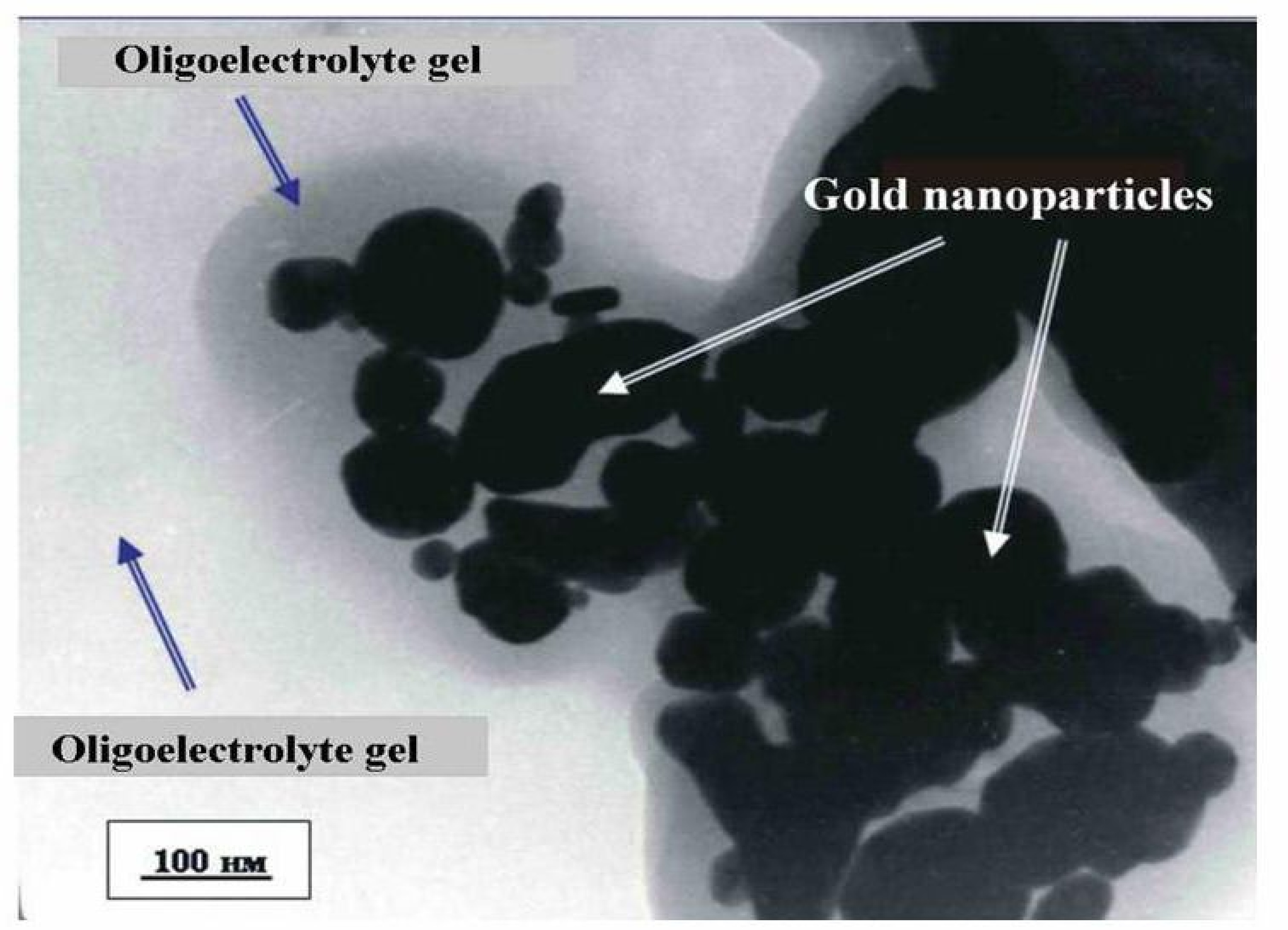


Luminescent spectrum of carboxyl-containing nanogel coordinated with Eu^{3+} cations. Excitation at 397nm (1); 387nm - (2); 300nm – (3).

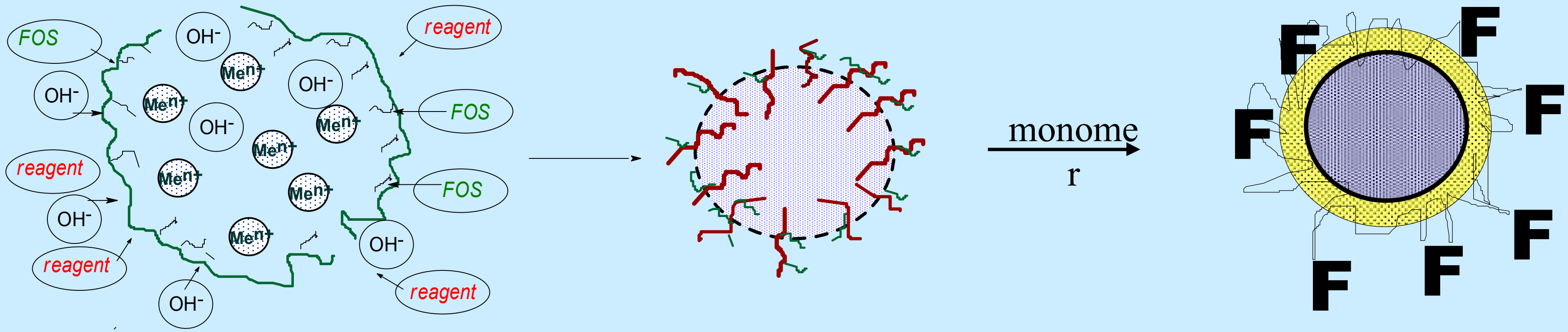


The scheme of the formation of luminescent carboxyl-containing nano gel carriers and loading of poor water soluble drugs

FUNCTIONAL NANOGEL FILLED WITH GOLD NANOPARTICLES



Homogeneous nucleation of metal, oxide, salt nanoparticles in the presence of oligoperoxide surfactants

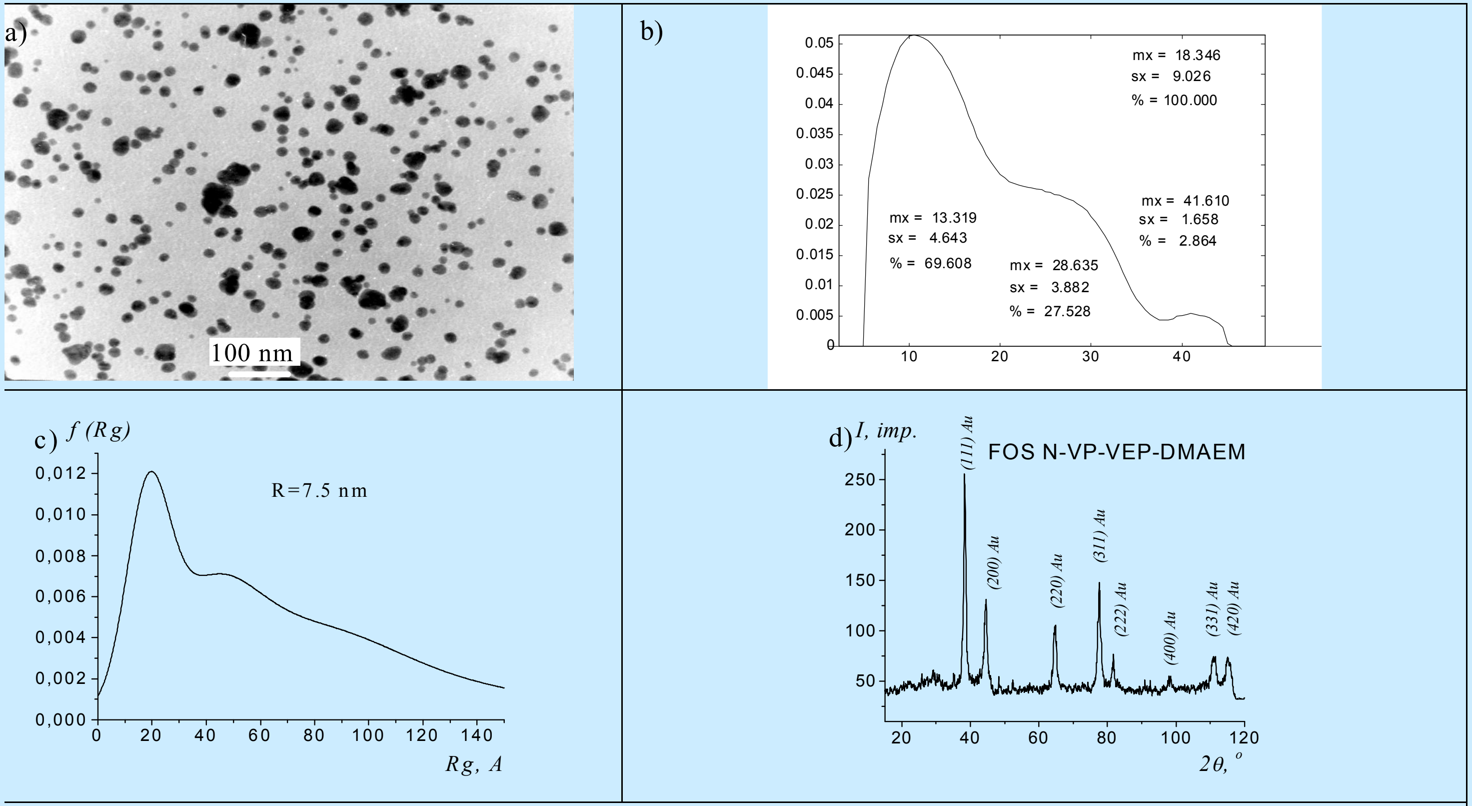


$$HAuCl_4 + 3NaBH_4 \rightarrow Au + 3B + 3H_2 + 3NaCl + HCl$$

$$4Ag(NH_3)^+ + N_2H_4 * H_2O + 4OH^- + 3H_2O \rightarrow 4Ag + N_2 + 4NH_3*H_2O$$

$$Cu^{2+} + 2HCHO + 4OH^- \rightarrow Cu + H_2 + 2HCOO^- + 2H_2O$$

Au nanoparticles with functional oligoperoxide shell



TEM micrograph (a), number-average size distribution (b, c) and XRD pattern (d) of gold nanoparticles obtained via chemical reduction from $HAuCl_4$ solution in the presence of oligoperoxide NVP-VEP-DMAEM. Particle size was calculated on the basis of TEM micrographs (b) and SAXS data (c)

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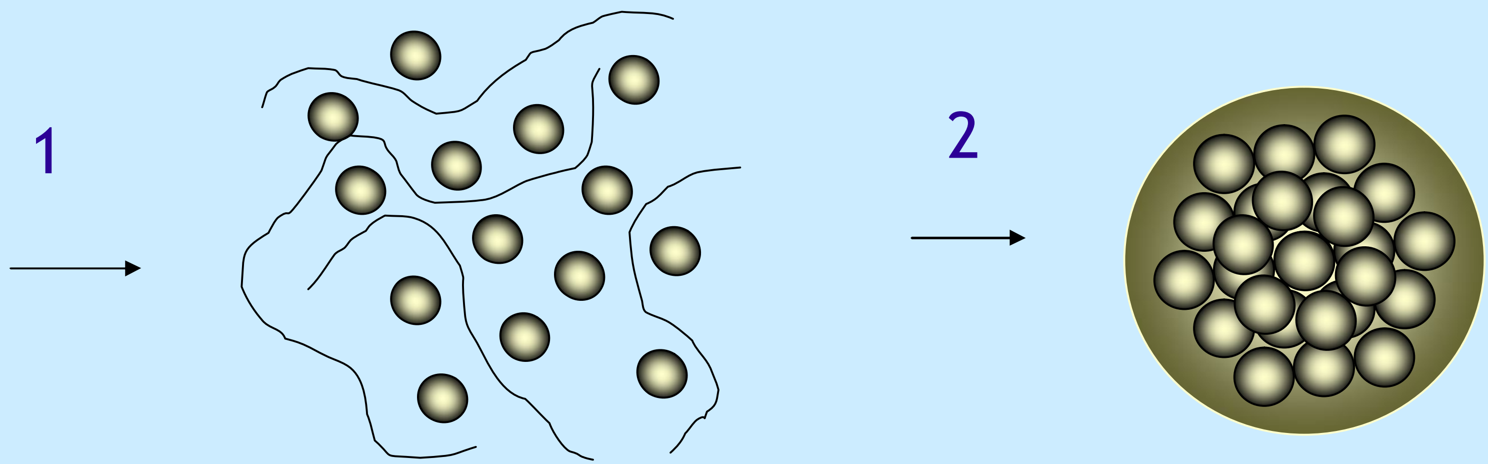
Magnetite nanoparticles with functional oligoperoxide shell

Scheme of functional reactive magnetite particles formation.

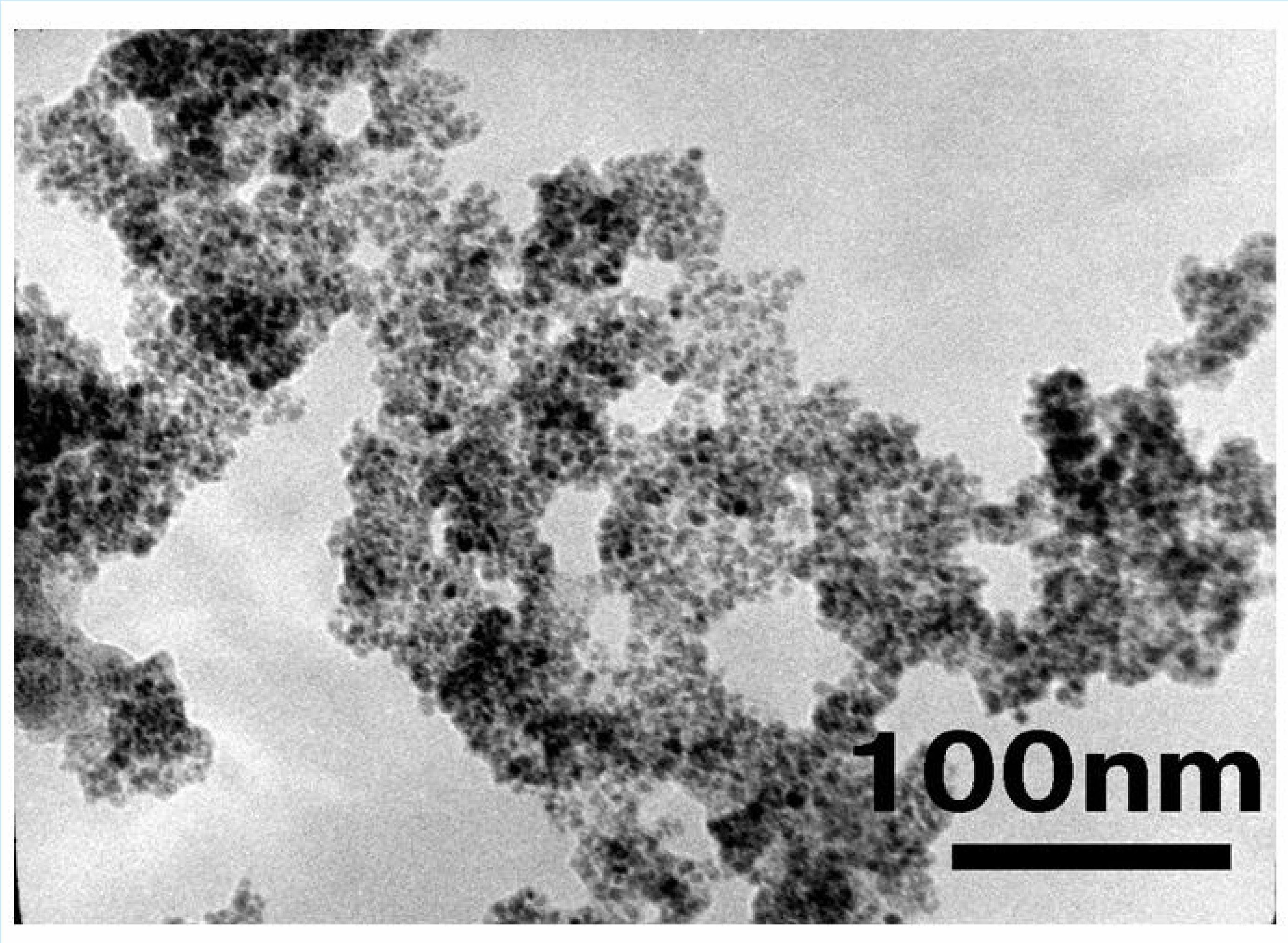
2FeCl3*6H2O + FeCl2*4H2O + 8NH4OH → Fe3O4 + 8NH4Cl + 20H2O

$2FeCl_3 \bullet 6H_2O$ +
$FeSO_4 \bullet 7H_2O$ +
$8NH_4OH$

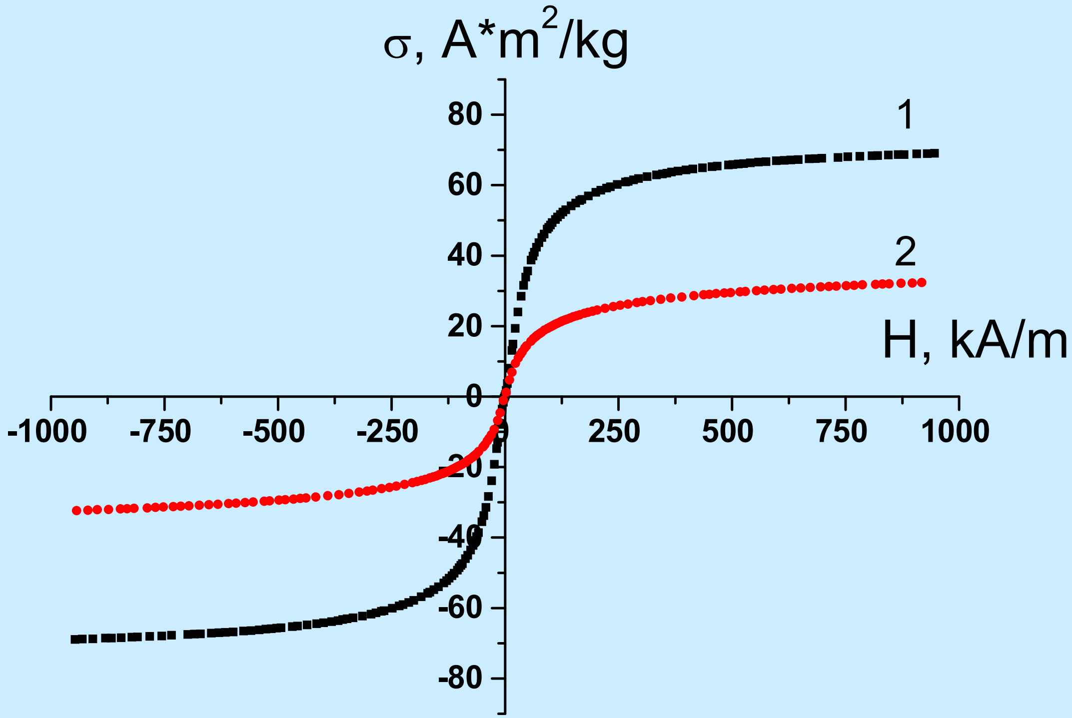


1) **Crystalline nucleation and growth in sites formed by oligoelectrolyte surfactant molecules**
2) **Crystalline binding with oligomer molecules and simultaneous aggregation resulting in formation of large particles with oligoperoxide sorption shell**

Magnetite particles properties. TEM, number-average crystalline size distributon. Large particles disaggregated after sonication



Magnetite particles properties. Magnetic behavior



1) Particles synthesized without modifier

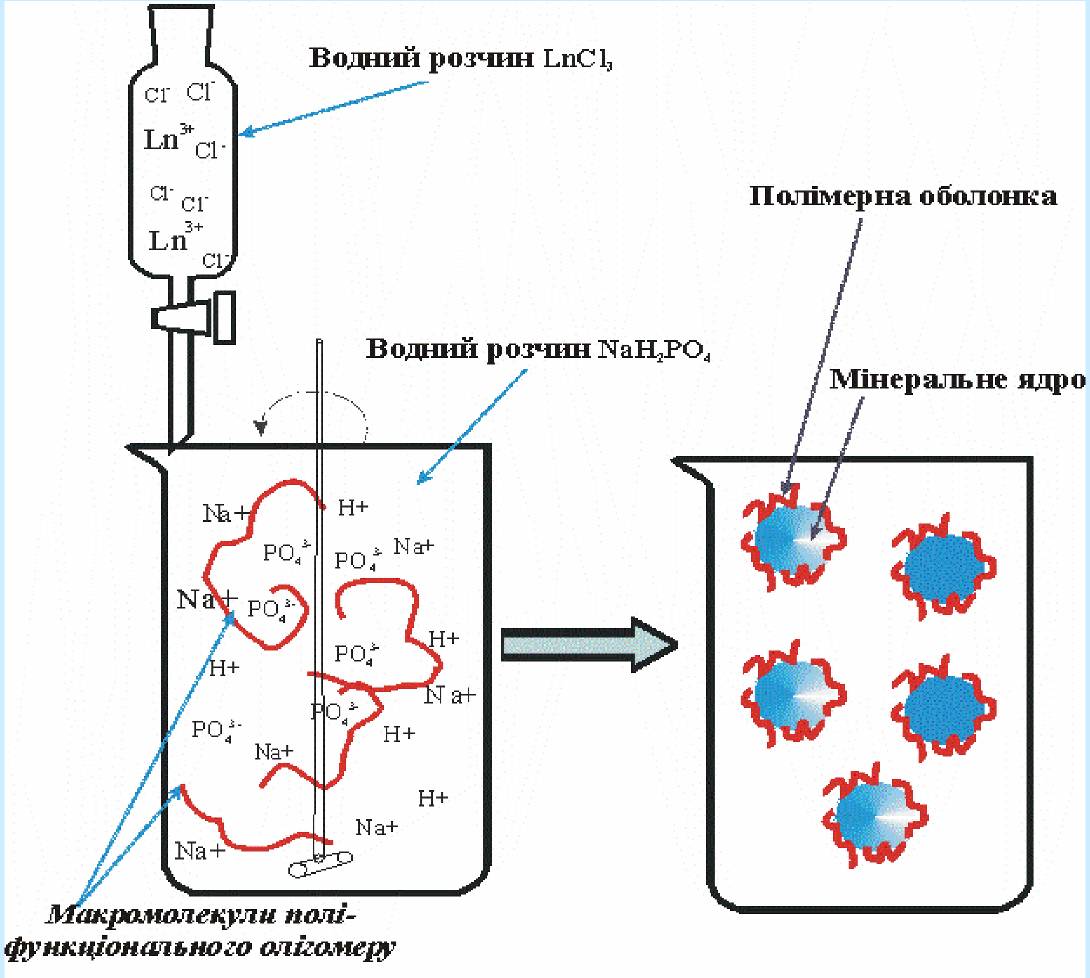
2) Particles synthesized with 2% oligomer

T = 293K

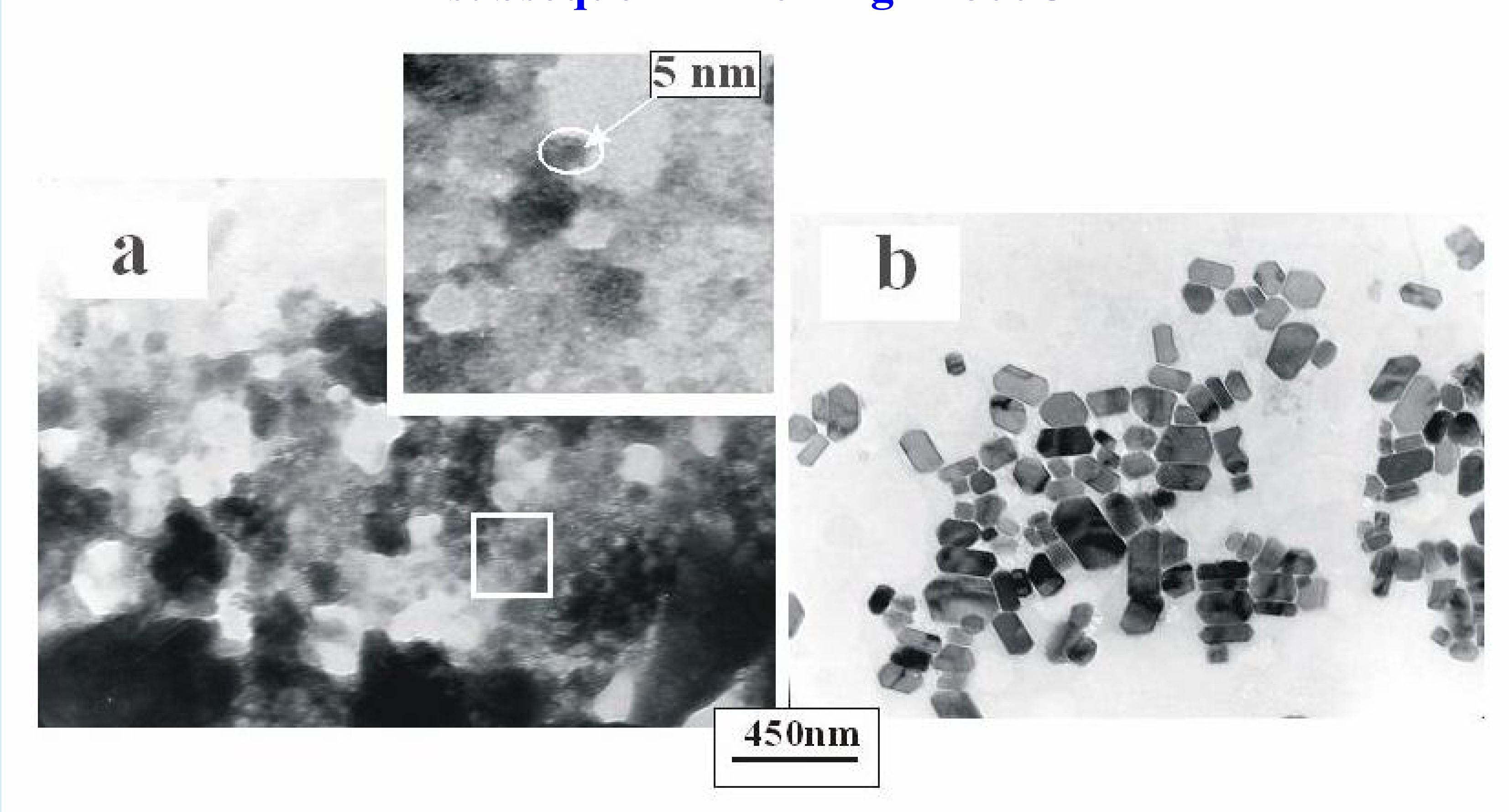
Superparamagnetic properties in spite of large size

The general scheme of micelle-sedimentation nucleation of LaPO4 nanoparticles

$$LnCl_3 + NaH_2PO4 \rightarrow LnPO_4\downarrow + 2HCl + NaCl$$



TEM images of $LaPO_4$ nanoparticles synthesized via micelle – sedimentation nucleation in the presence of 2.5% VA-VEP-MAN oligoperoxide as well as after subsequent annealing at 800C



a – initial nanoparticles; b – after annealing at 800C (magnification 30000)

Characteristics of the adsorption of oligoperoxide surfactants onto $LaPO_4$ nanoparticle surface

Surface modifier	Mn, g/mole	Concentration of modifier in solution, %	Particle size,nm	Particle surface area, nm^2	Content of modifier on particle surface, %	Surface area per modifier molecule on particle surface,nm^2
Ricynox-80	724	1,0	9,0	254,3	0,9	278,0
		2,5	9.1	260,0	1,2	207,0
		5,0	9,0	254,3	1,2	209,0
		7,0	8.9	248,7	1,2	211,0
NVP-VEP-GMA	3200	1,0	8.9	248,7	1,5	700,0
		2,5	8.5	226,9	3,2	344,0
		5,0	7.7	186,2	20,0	60,7
		7,0	7.5	176,6	21,0	59,3
VA-VEP-MAN	2000	0,1	7,0	153,9	2,0	445,0
		0,5	6.8	145,2	3,8	241,0
		1,0	5,0	78,5	8,6	145,0
		2,5	4.9	75,4	18,6	68,3
		5,0	4.6	66,4	25,0	54,2

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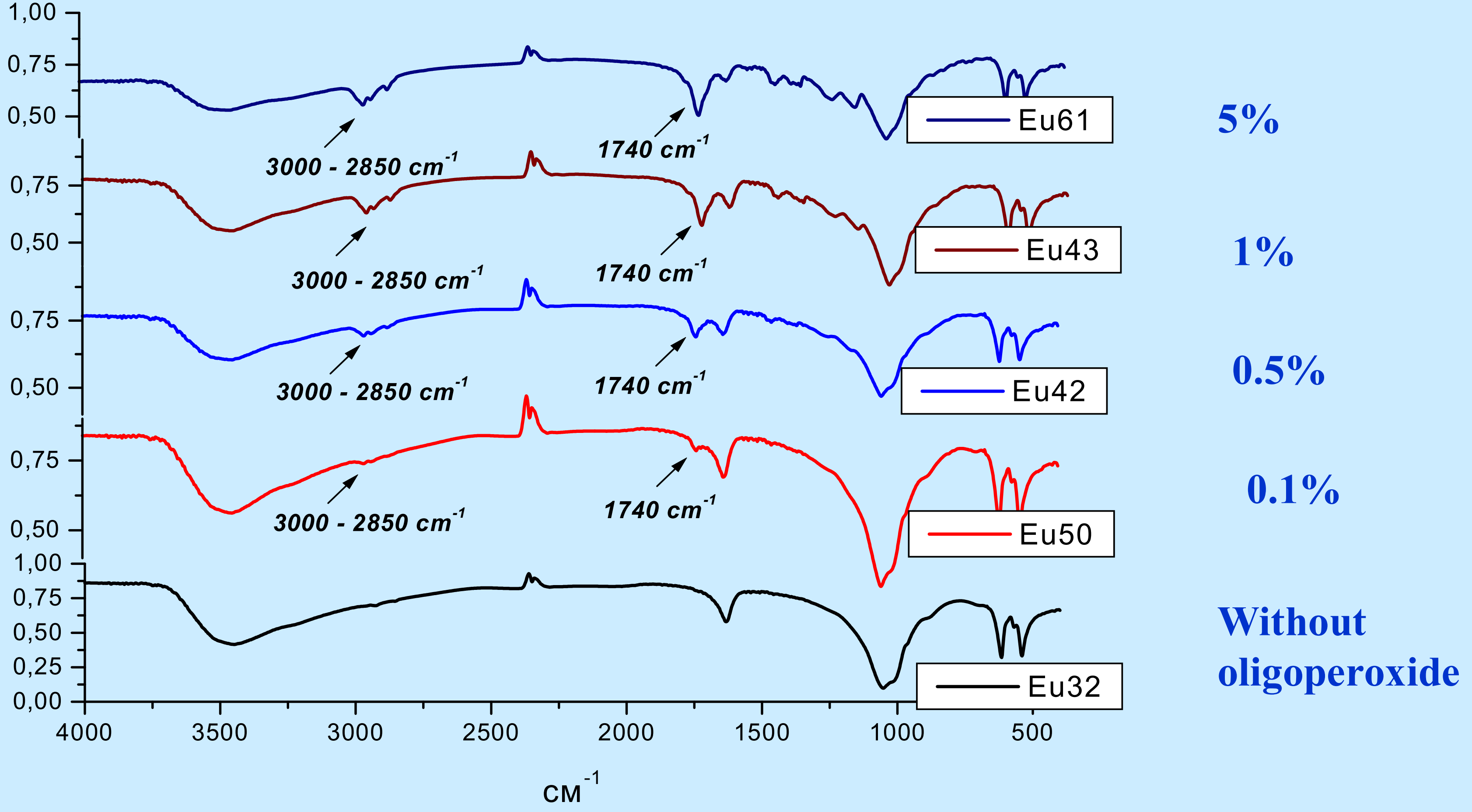
FT-IR spectrum of functional $LaPO_4$ nanoparticles synthesized and modified with VA-VEP-MAN oligoperoxide surfactant



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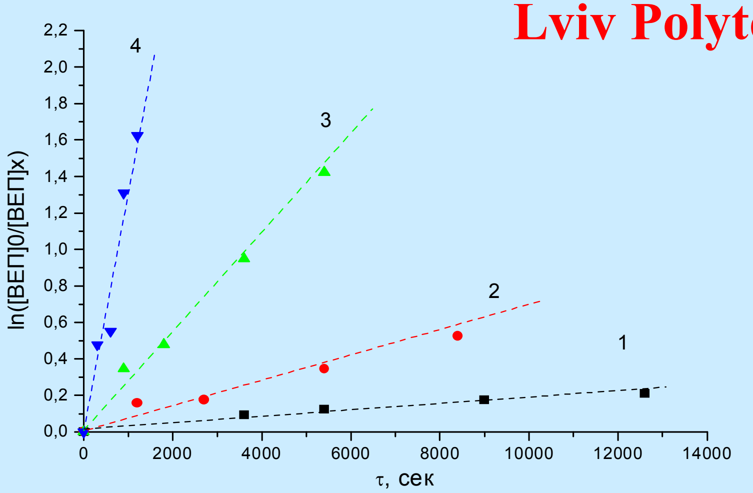


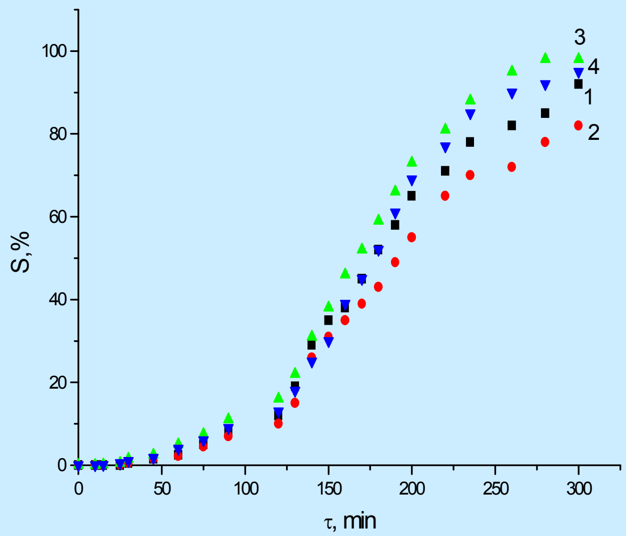
Semi logarithmic anamorphous of kinetic curves of decomposition of VEP-links of oligoperoxide N-VP, VEP, GMA on в діоксані при $LaPO_4$ particle surface at: 1 – 373К; 2 – 393К 3 – 423К, 4 -453К

Coefficients of the rates of thermal decomposition (Kd) and induced decomposition (Ki) of peroxide groups in oligoperoxides NVP-VEP-GMA and VA-VEP-MAN immobilized on $LaPO_4$ nanoparticle surface(423К)

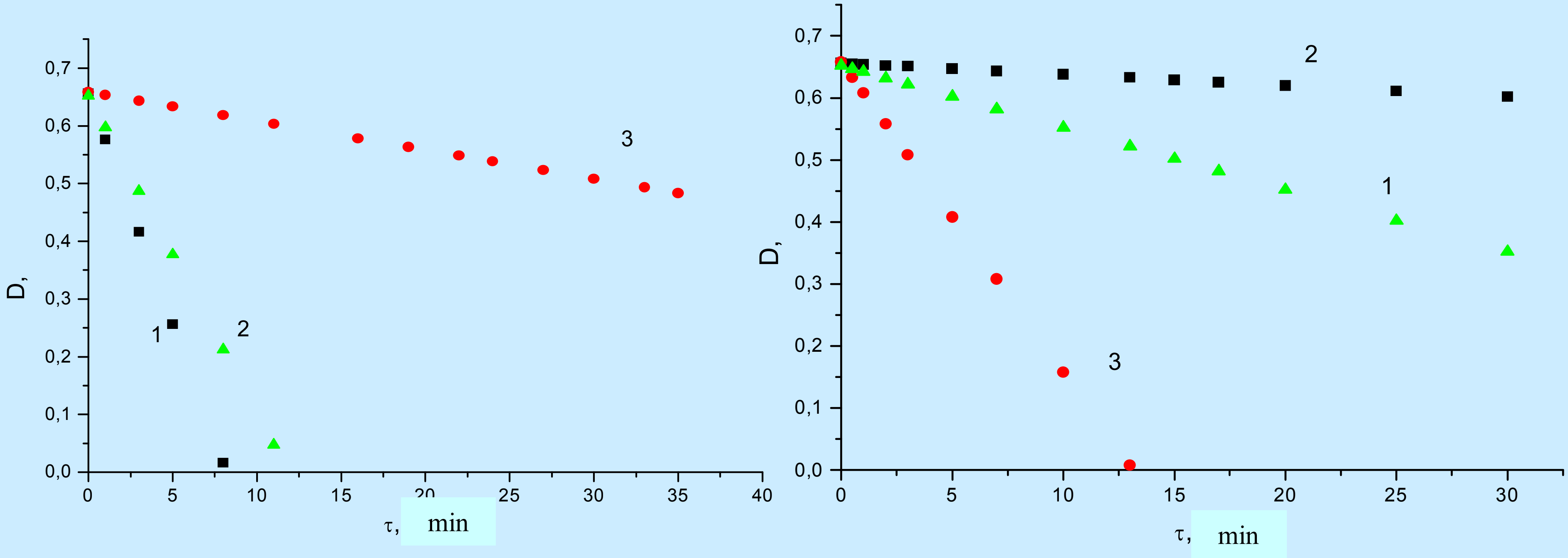
$LaPO_4$ coated by oligoperoxide NVP-VEP-GMA				$LaPO_4$ coated by oligoperoxide VA-VEP-MAN			
[VEP-links], mole/l	$K_{еф\text{-}d} \cdot 10^5$, s^{-1}	$K_i 10^5$, l/mole·s	$K_d 10^5$, s^{-1}	[VEP-links], mole/l	$K_{еф\text{-}d} \cdot 10^5$, s^{-1}	$K_i 10^5$, l/mole·s	$K_d 10^5$, s^{-1}
0.008	20.0	1100.0	11,2·	0.047	89.0	-	-
0.015	25.0			0.139	90.0		
0.023	40.0			0.207	87.0		
0.030	43.0						

Characteristics of nanoparticles after graft radical polymerization ($LaPO_4$•Eu (3.5%) coated by oligoperoxide VA-VEP-MAN)

Content of oligoperoxide on initial particle surface, %	Monomers	Content of monomers per particles, %	Content of polymeric shell, %	Content of grafted polymer, %
17.0	NVP: GMA (70:30 moles)	30	38.0	21.0
17.0		50	44.5	27.5
16.5	Styrene	30	30.0	13.5
16.5		50	43.0	26.5



The dependence of the conversion degree of styrene (3,4) and mixture NVP - GMA (1,2) on time of water dispersion polymerization initiated from $LaPO_4$ particle surface; monomer content 30% (1, 3) and 50% (2, 4) per $LaPO_4$, (70C, nanoparticles: water =1:5.)



The dependence of the transmission density of LaPO4•Eu nanoparticle suspensions in toluene (a) and alcohol (b) on time. 1 – nanoparticles synthesized via micelle-sedimentation technique in the presence of NVP-VEP-GMA; 2 –nanoparticles coated by graft copolymer of NVP-VEP (80:20); 3 - nanoparticles coated by polystyrene.

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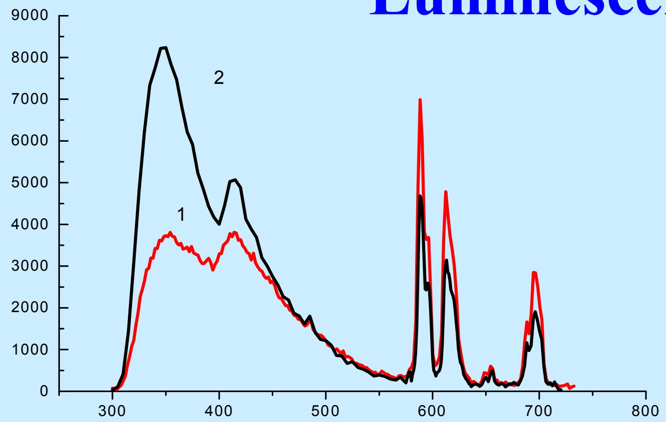
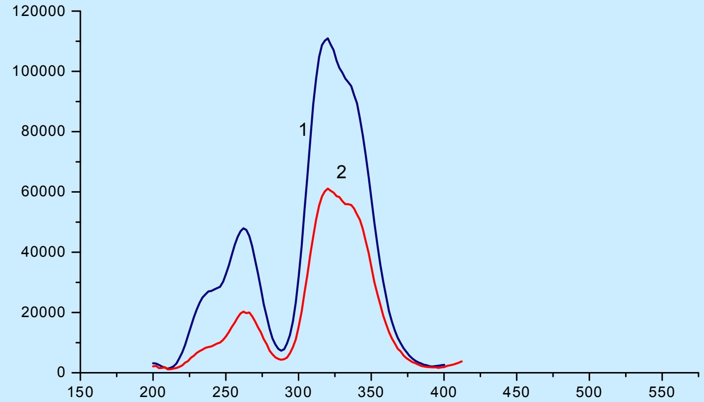
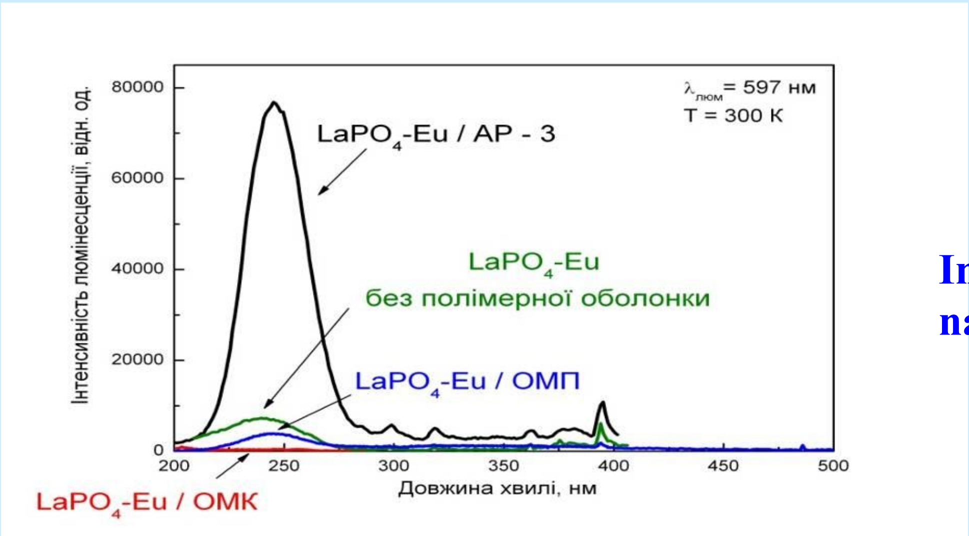
Luminescence and scintillation spectrum



$LaPO_4$•Eu nanoparticles synthesized in the presence of oligoperoxide VA-VEP-MAN (1), and after graft polymerization of NVP-GMA mixture (2). X-ray excitation



$LaPO_4$•Pr nanoparticles annealed (1) and after following sorption activation by oligoperoxide and radical grafting polystyrene shell (2). X-ray excitation



Influence of oligoperoxide shell nature immobilized on nanoparticle surface on luminescence intensity

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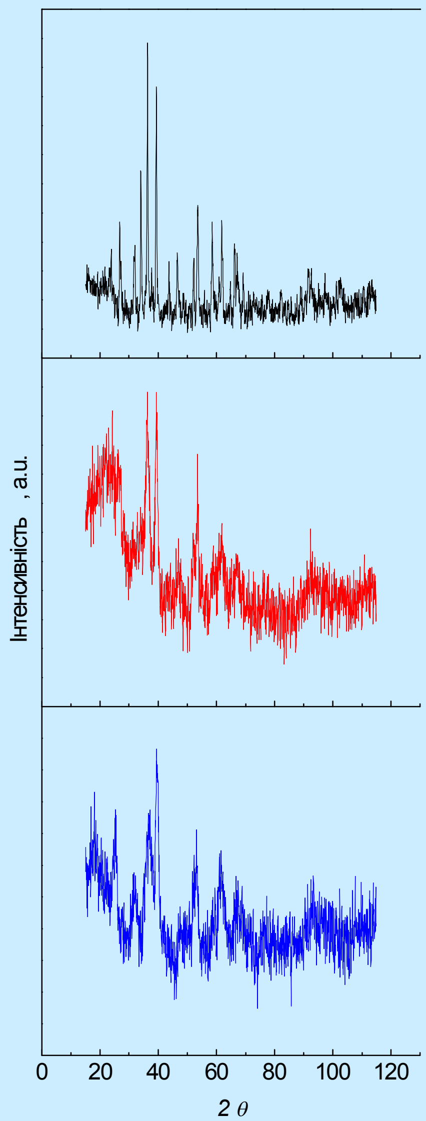
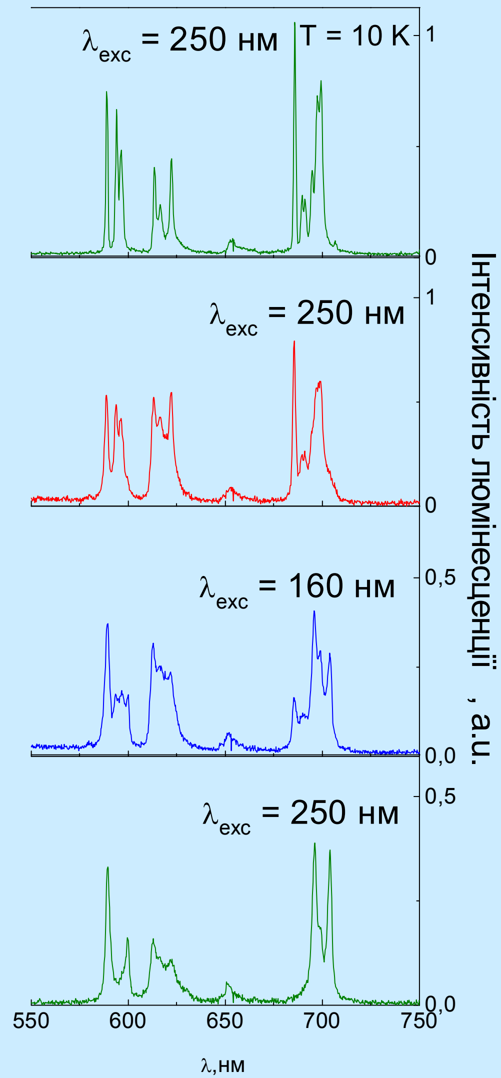
X-ray patterns and luminescence spectrum of $LaPO_4$•Eu nanoparticles annealed at distinct temperatures



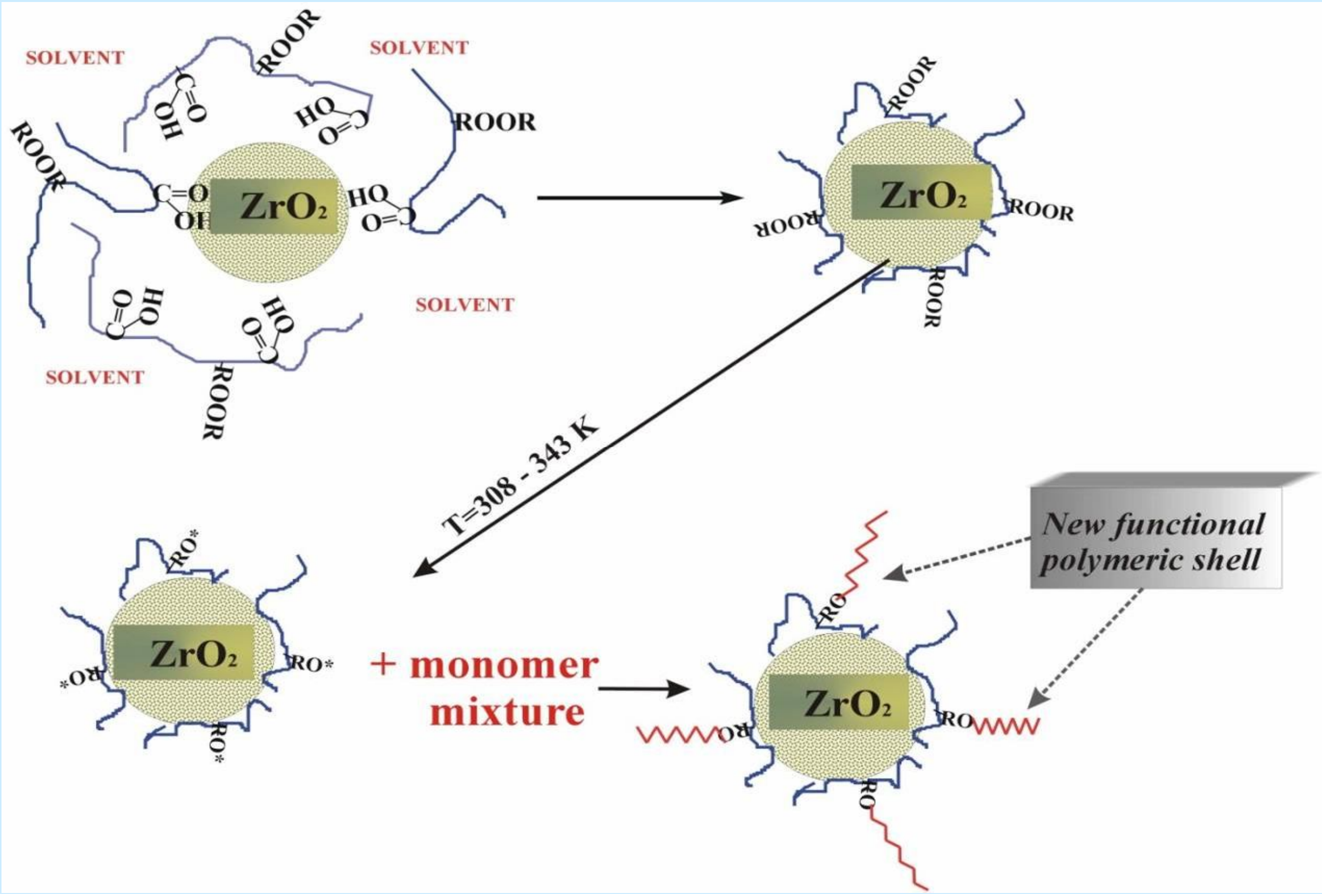
$LaPO_4$·Eu, annealed at 800C; monoclinic structure of lattice, size ~70nm
$LaPO_4$• Eu, annealed at 400C; monoclinic and hexagonal structure of lattice, size ~12nm
$LaPO_4$• Eu, non annealed; hexagonal structure of lattice, size ~5 -7nm



49

Sorption modification of polymer and mineral nanoparticles

Sorption modification of ZrO_2 nanoparticles (30nm) doped by Eu (3+) cations with oligoperoxide surfactants and following radical grafting of functional chains



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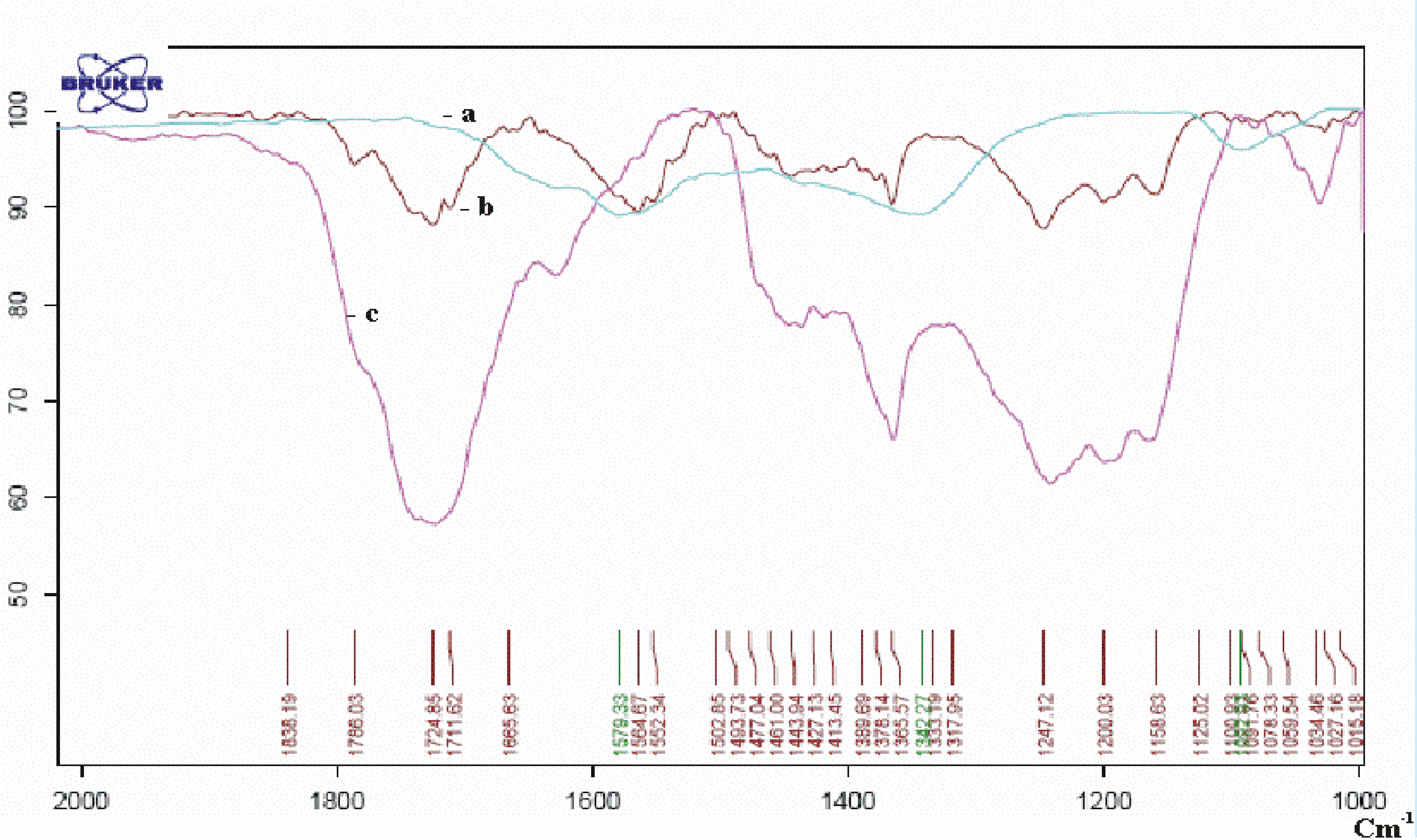
FT-IR spectra of ZrO_2 (3% of Y_2O_3) – a; of ZrO_2 (3% Y_2O_3) modified with oligoperoxide metal complex – b; oligoperoxide metal complex - c.



Why such oligoelectrolyte based nanoparticles?

•Controlled particle size and size distribution

•Controlled functionality and reactivity

•Reactions initiated from the surface

•Peroxide links homolysis in the range 298 - 343 K with free radical formation.

•Presence of peroxide links on particle surface provides tailored particle functionalization (epoxide, aldehyde, maleimide etc.) via graft copolymerization.

•Availability of controlled reactive functionality on nanoparticle surface provides attachment of cell recognizing biological vectors (saccharides, lectins, antibodies).

3. Cellular studies and potential biomedical application

3.1. Cell tagging with functional nanoparticles for their visualization (microscopic or imaging study) and isolation

3.2. Engulfment of functional biocompatible luminescent, coloured and magnetic polymeric and hybrid nanoparticles by the cells

3.1. Cell tagging with functional nanoparticles for their visualization (microscopic or imaging study) and isolation

Optically Dense Markers:

functional gold, silver, nickel, magnetite, maghemite, and polymeric nanoparticles coloured by unsaturated dyes and fluorescein in the core etc.

Luminescent Markers:

functional luminescent polymers and polymeric and hybrid nanoparticles containing coordinated rare earth elements Ce (III), Eu (III) in the core or shell

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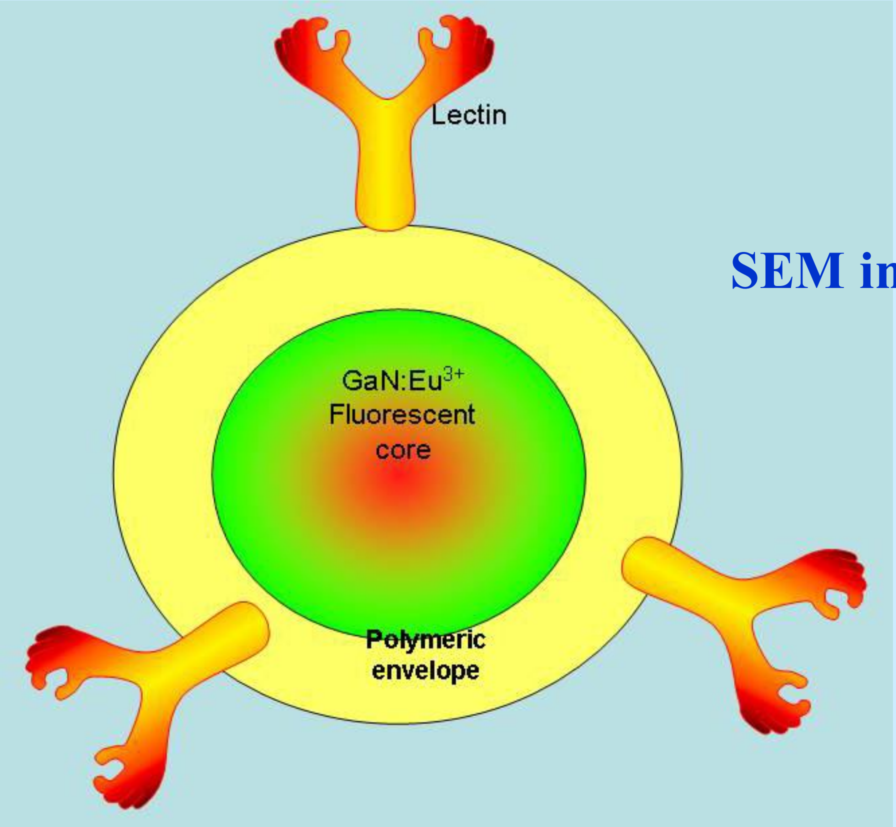
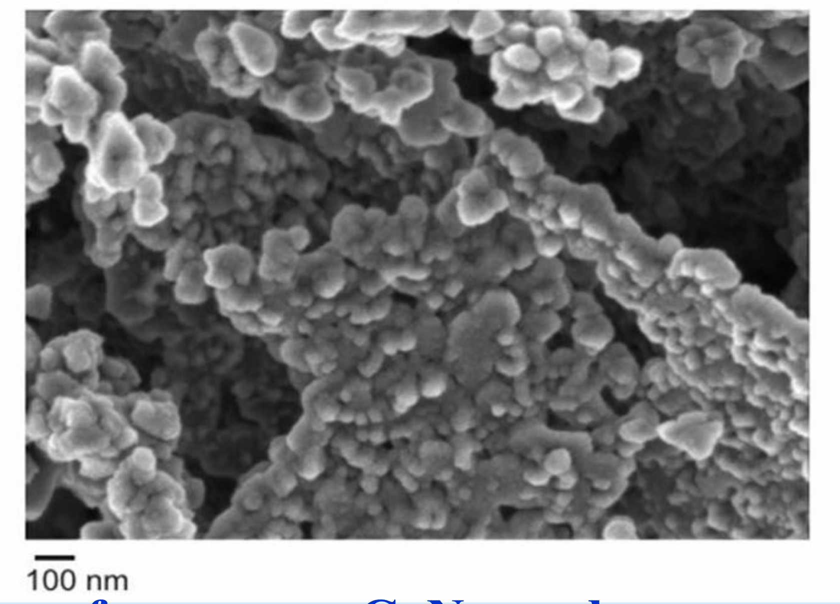
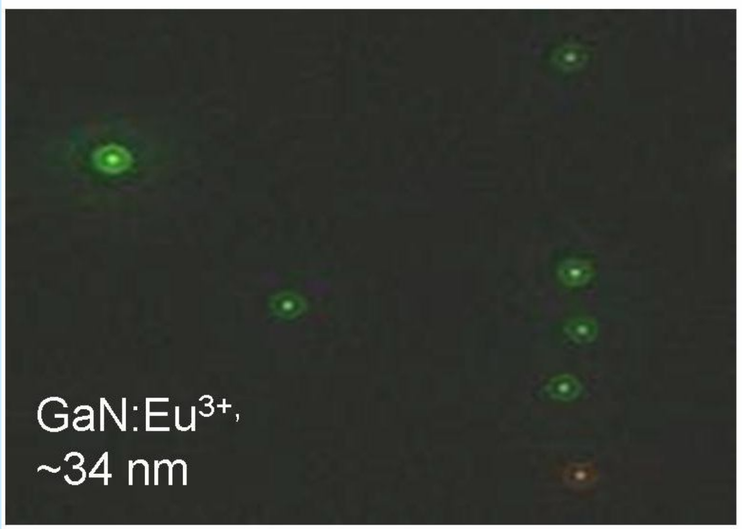
$GaN:Eu^{3+}$-PSL lectin conjugated nanoparticles





SEM image for pure nc-GaN powder



Fluorescence of $GaN:Eu^{3+}$ nanoparticles

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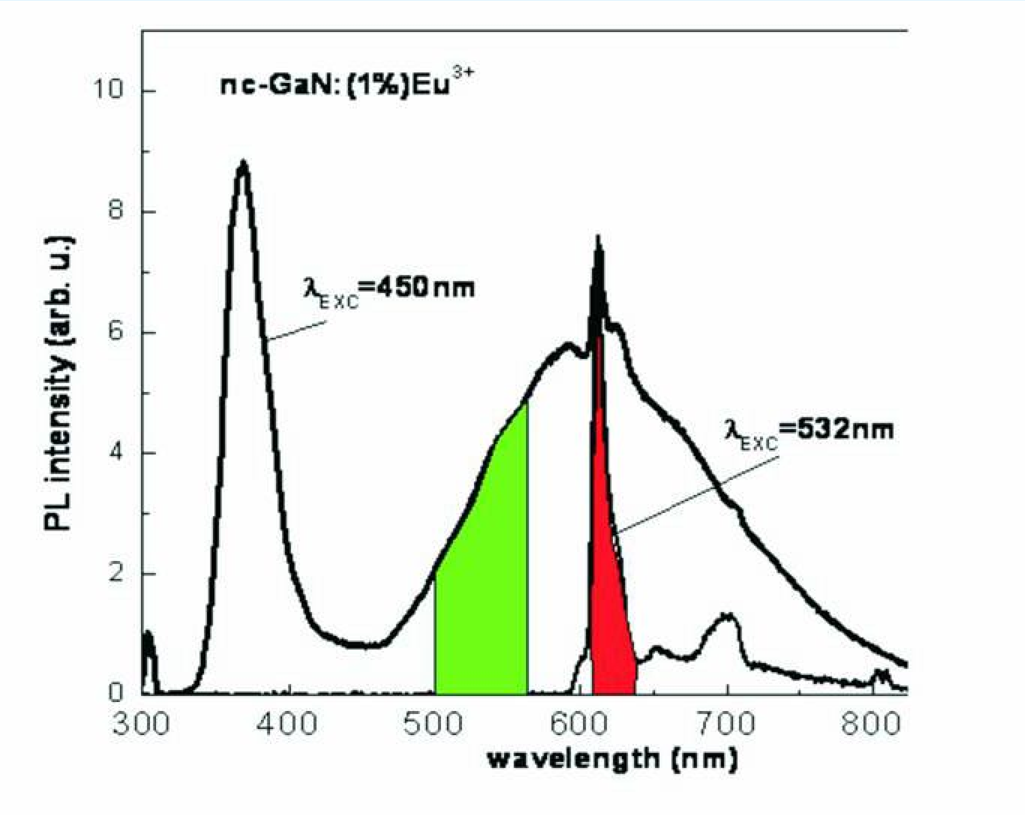
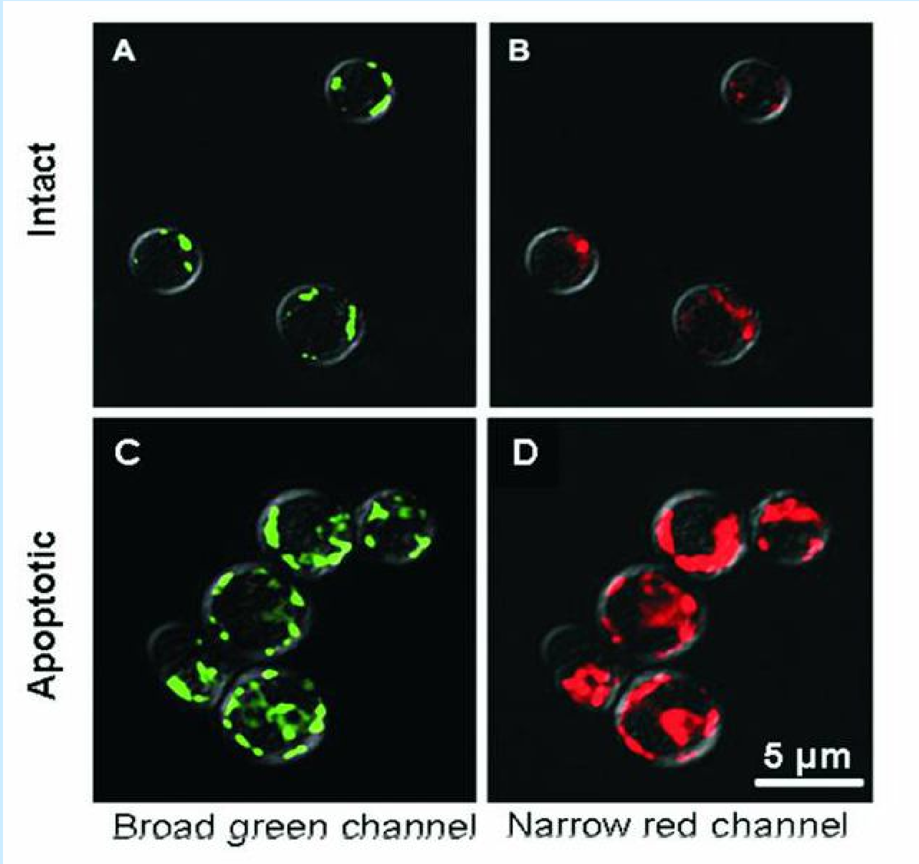
Bioconjugated nanoparticles GaN:Eu3+-PSL lectin specifically bind to apoptotic cells





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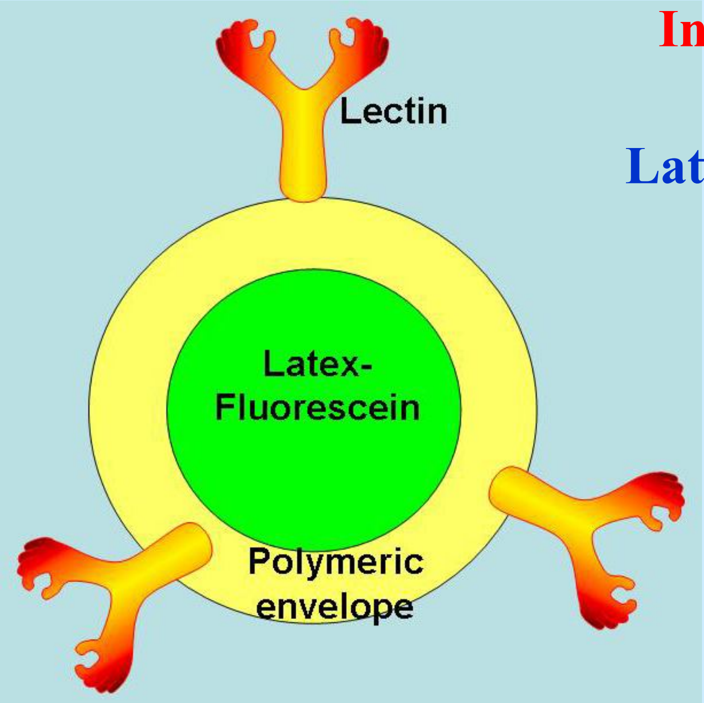
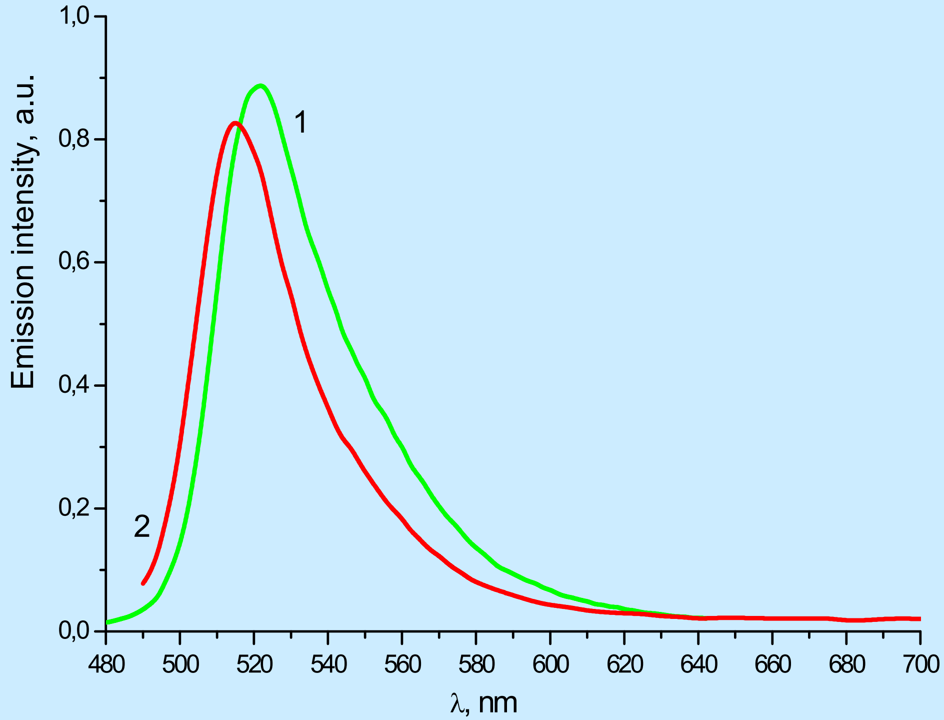
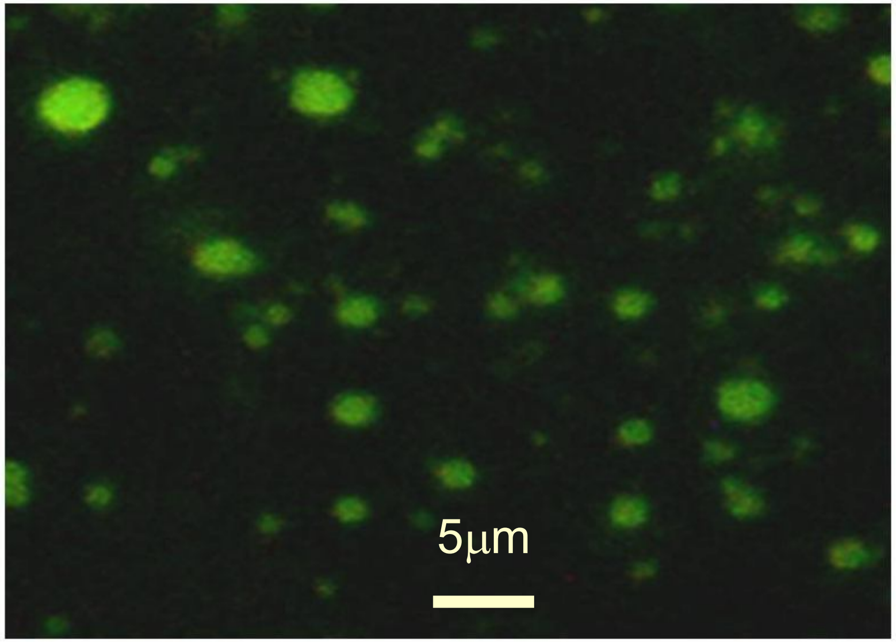


Latex-fluorescein-lectin conjugated nanoparticles





Emission spectra of fluorescein (1) and fluorescein-containing latex nanoparticles (2); excitation at 425 nm.

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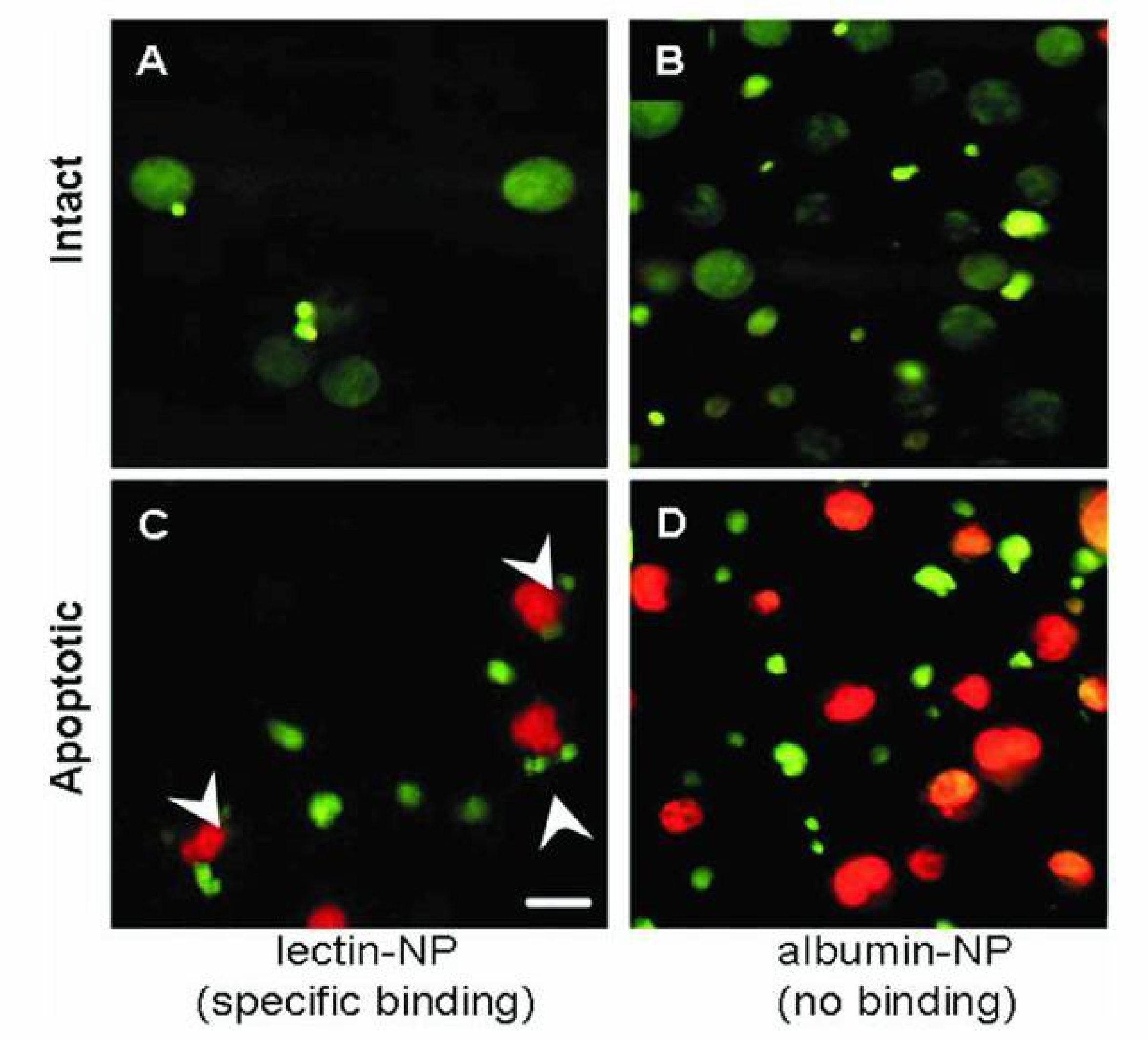
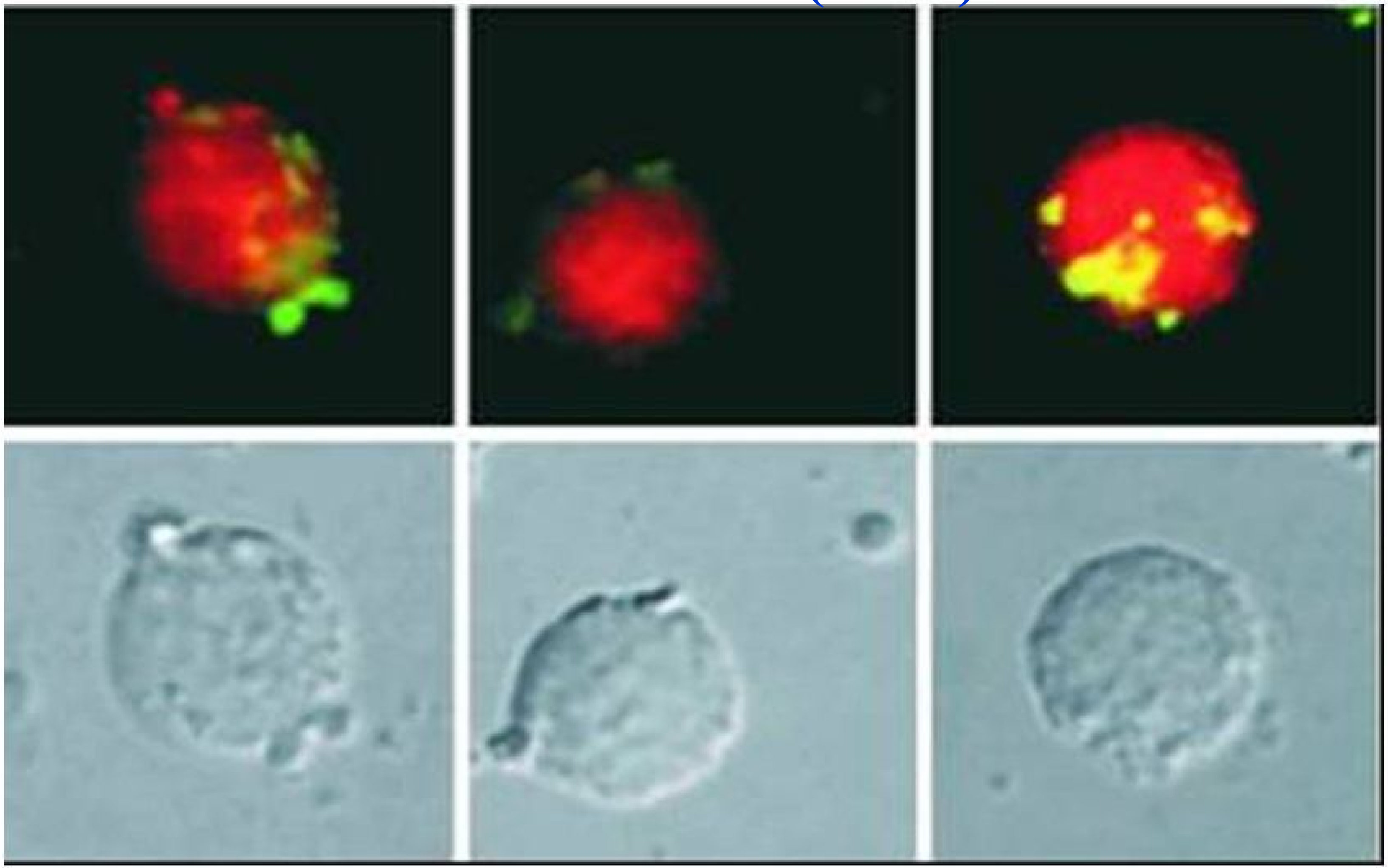
Bioconjugated fluorescein-containing WGA-lectin-conjugated nanoparticles used for the detection of necrotic cells



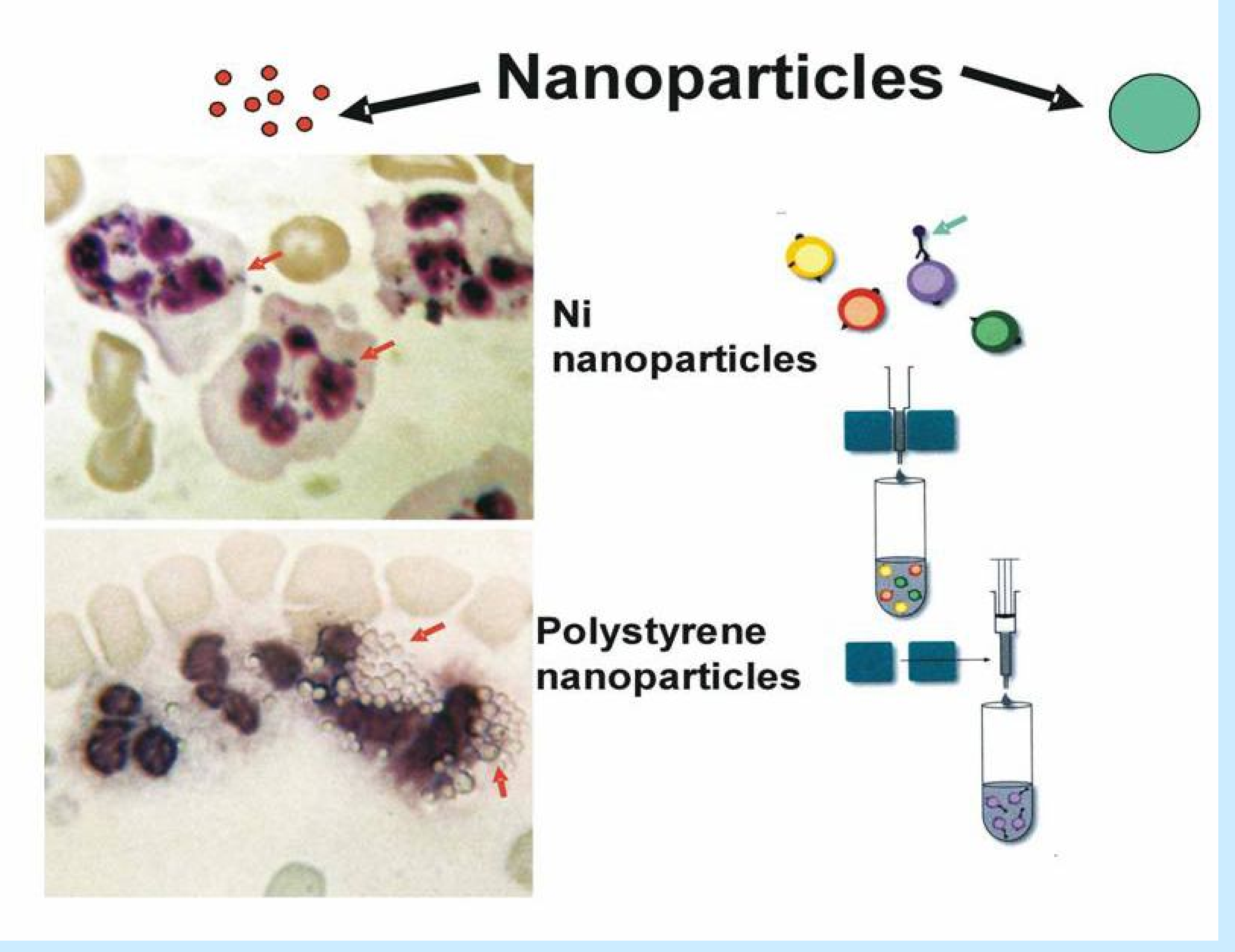
In A and B living cells are counterstained with 1:100,000 (w/v) acridine orange solution (fain green), in C and D dead cells are counterstained with propidium iodine (1 |jg/ml) solution to visualize nuclei of dead cells (red). White bar correspond to 5 µm

DOUBLE STAINING OF MURINE T LYMPHOCYTES WITH FLUORESCEIN NCS CONJUGATED WITH ANTI-TCR (GREEN DOTS) OR ANTI-CD3-TEXAS RED ANTIBODY (RED).

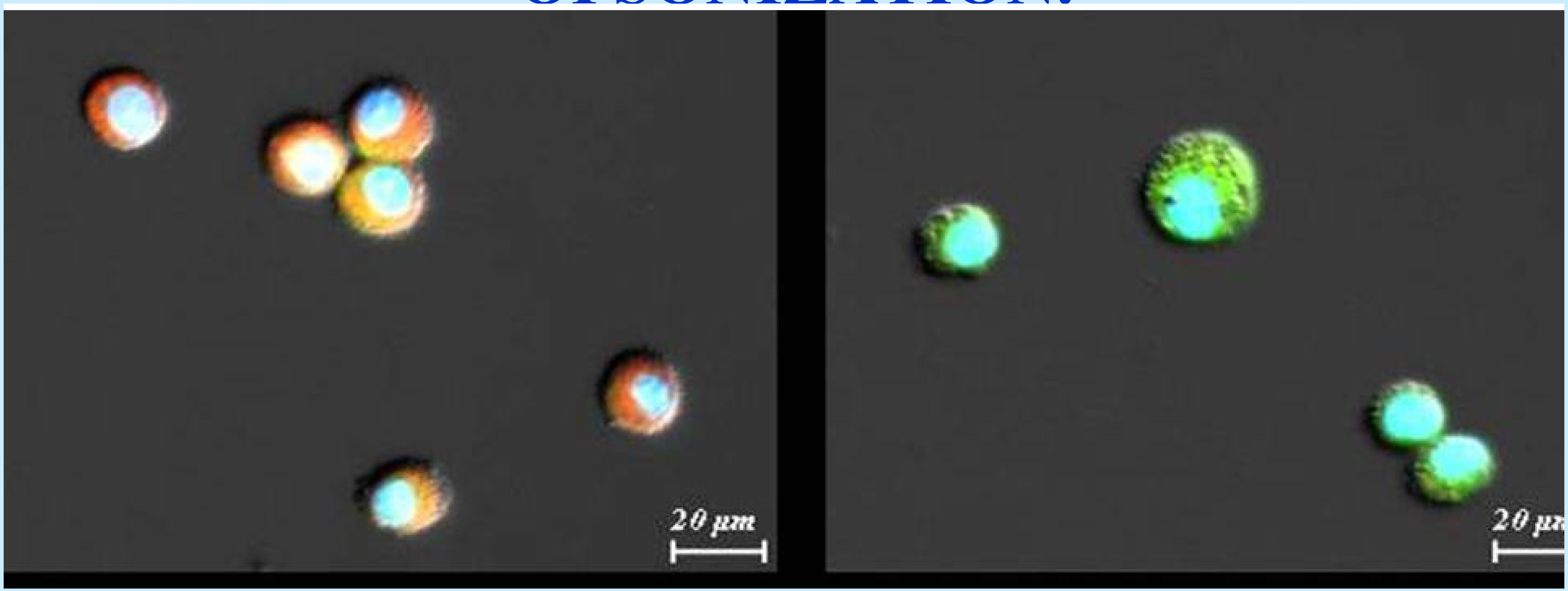


3.3. Engulfment of functional biocompatible luminescent, coloured and magnetic polymeric and hybrid nanoparticles by the cells

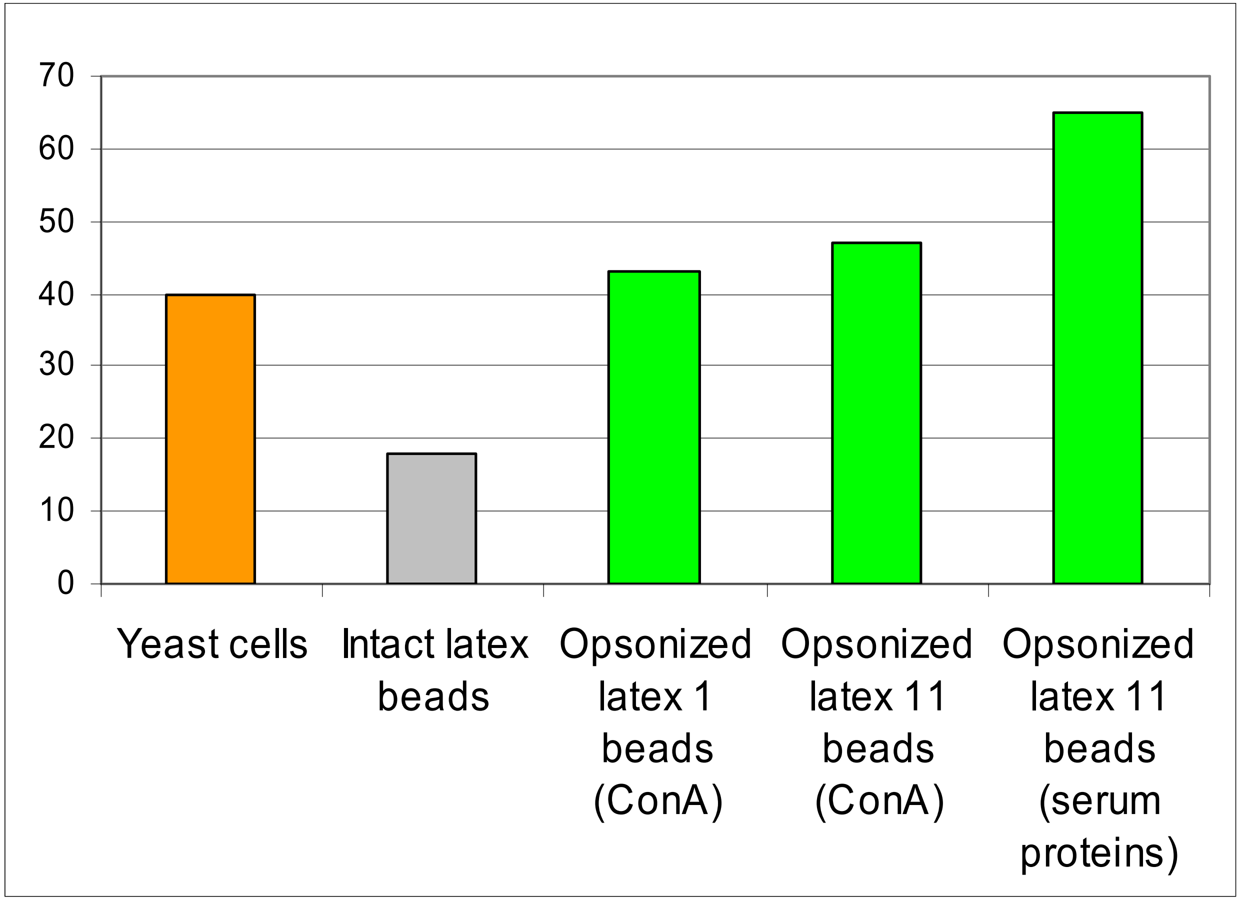
Institute of Cell Biology



MURINE MACROPHAGES OF J774.2 LINE TREATED WITH MAGHEMITE NANOPARTICLES: A – LACKING POLYMERIC SHELL AND PROTEIN OPSONIZATION; B – WITH POLYMERIC SHELL AND PROTEIN OPSONIZATION.



Biofunctionalization of functional polystyrene nanoparticles by proteins recognizing cells



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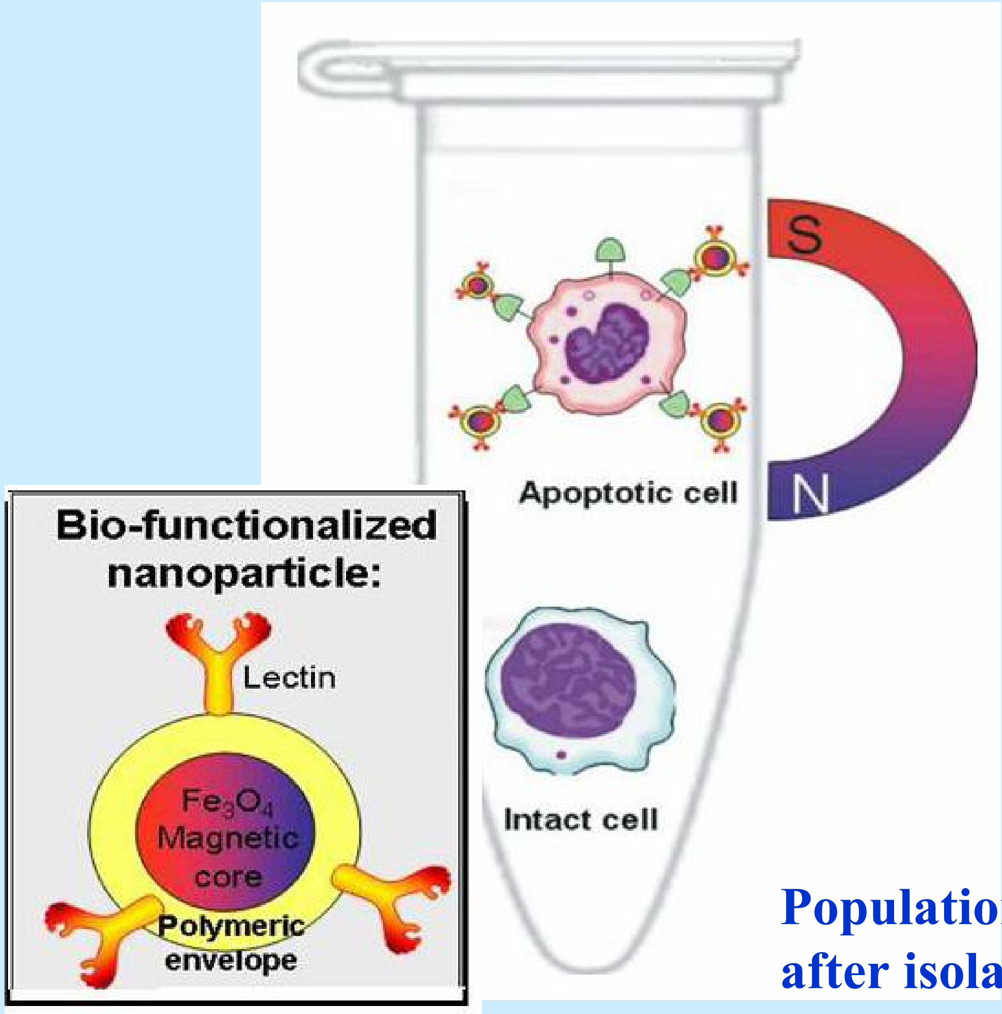
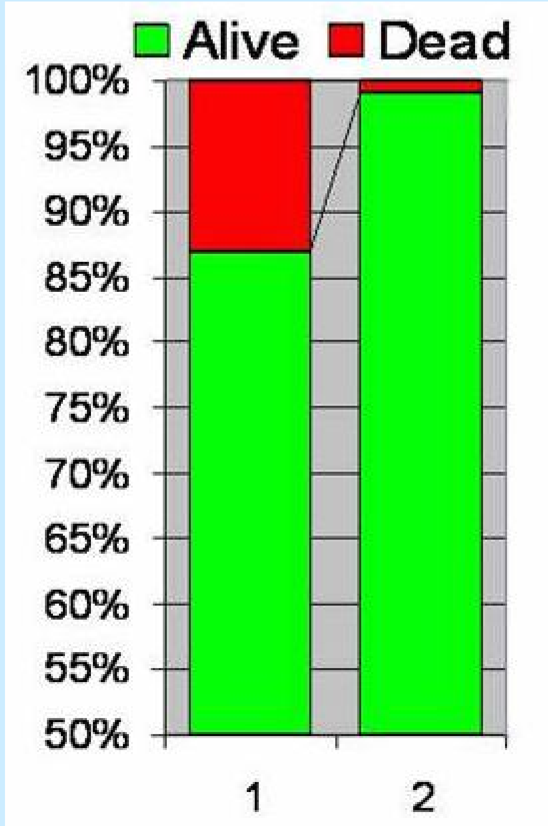
Isolation of pathological cells using functional lectin modified nanoparticles

Development of lectin-biofunctionalized super paramagnetic nanoparticles

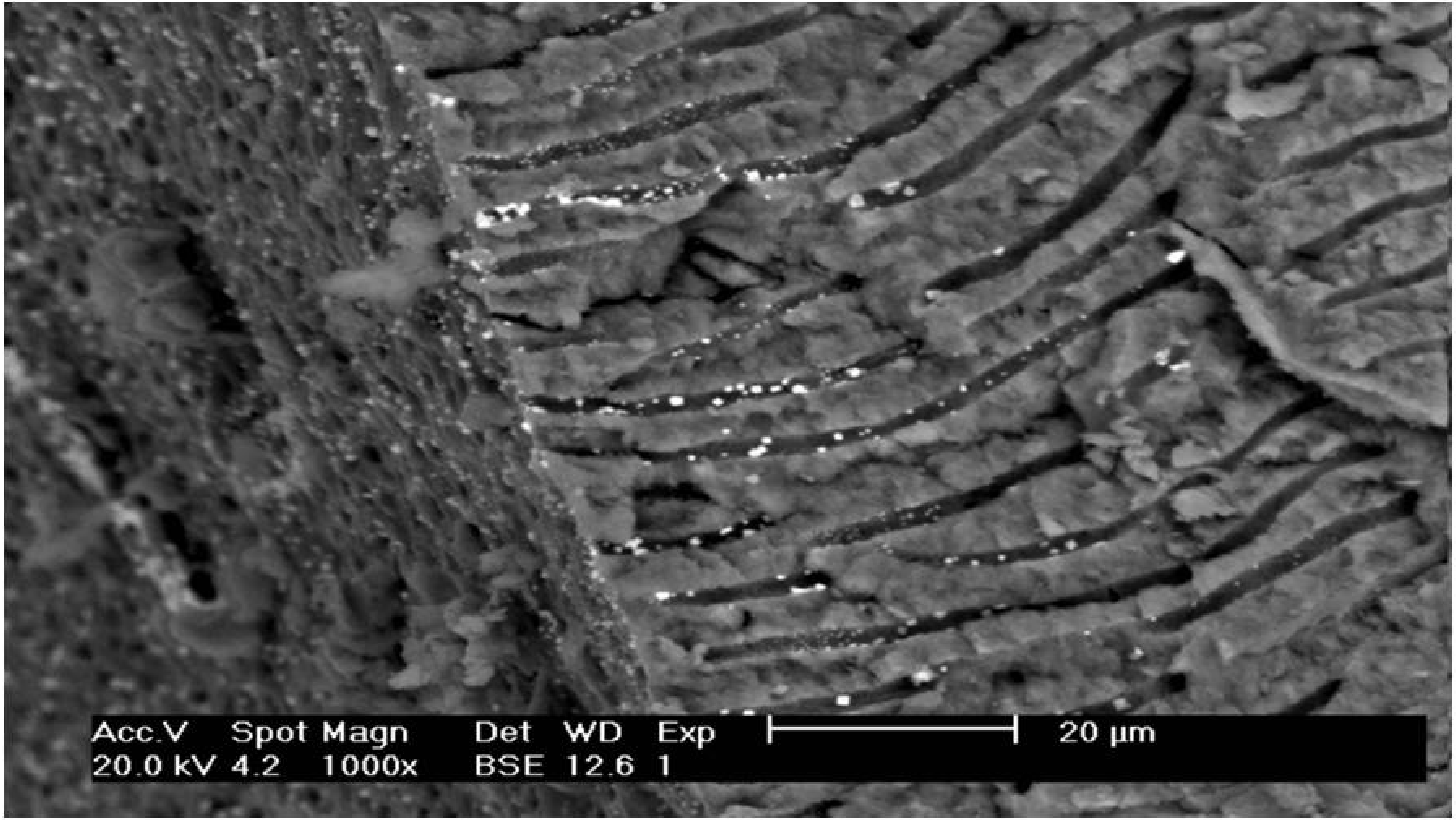




Population of Jurkat T-cells before (1) and (2) after isolation of dead cells using HHA lectin-conjugated Fe304 nanoparticles.

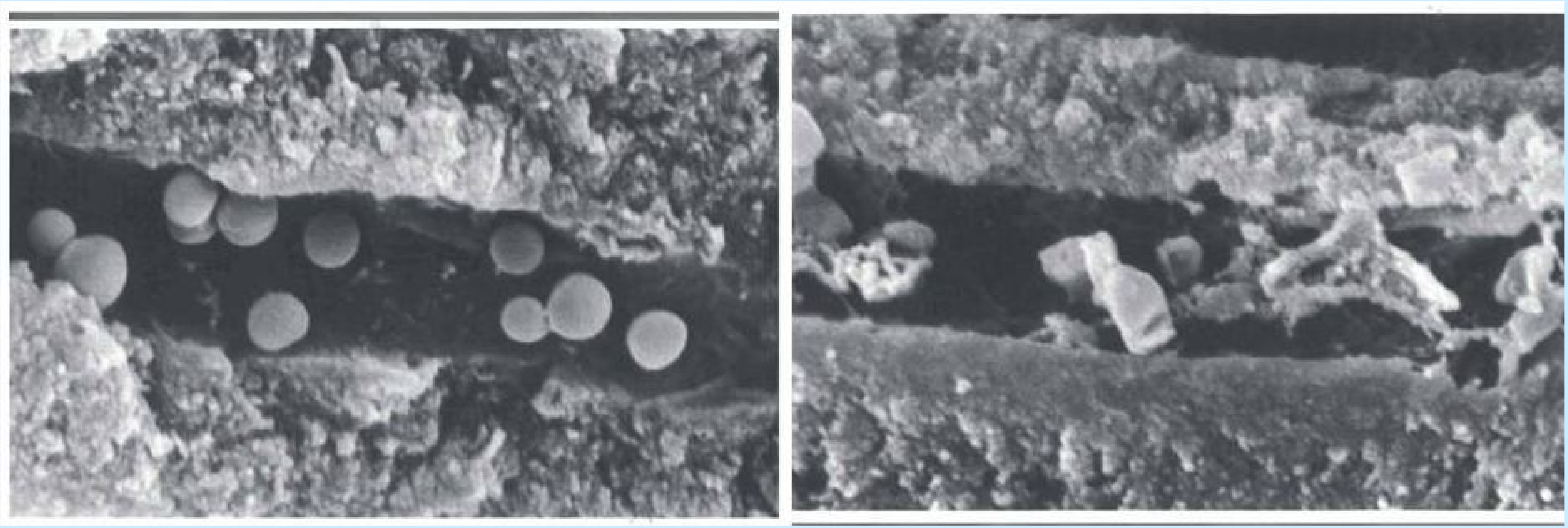
Vienna Technical University

Silver nanoparticles in the tooth channels



Vienna Technical University

Micrographs of tooth channel before (a) and after (b) treatment with silver hydrosol



a) b)

5. Acknowledgements

Many thanks to my team, colleagues and partners

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- **Professor Ya. Bobitsky, Head of the Department of Photonics, Lviv Polytechnic National University etc.**
- **Professor A. Voloshinovsky, Head of the Department of Experimental Physics, I. Franko National University;**
- **Dr. Ya. Khimyak and team, Liverpool University, UK**
- **Dr. D. Horak, Institute of Macromolecular Chemistry of Czech Academy of Sciences, Prague, CzR**
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and

thank you for your attention!