CHALLENGES FOR TOXICOLOGY AND RISK ANALYSIS OF BIONANOMATERIALS IN 21 CENTURY

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Linking xenobiotic chemical exposure to health effects and diseases has been the subject of many experimental and epidemiological studies, though this issue remains a matter of permanent discussion and controversy. One major issue in linking chemical exposure to health problems refers to choice of biomarkers. This issue is complicated by the multiple mechanisms of pesticide toxicity often involved, the uncertainties related to long term and low dose pesticide exposure, and the reliable identification of exposed and control groups. The characteristics of exposure as duration and involvement of variable environmental factors and mixtures in epidemiological studies and biomonitoring data for xenobiotics low level long-term exposure together with analytical problems related to these studies will be investigated and evaluated in a brief overview of published studies. Exposure scenarios simulating real life is a complex issue and effects from multiply chemicals must be considered as a web of interactions that produce variety mechanisms and subsequently of effects. In this respect liner -monomodal but aslo non linear effects can be seen in the range of low and/or high concentrations of exposures. Evaluating exposure effects is considered a multifactorial task that needs an integrated and systematic approach not only for long term actions but often for acute or sub chronic actions. Real life is a variability and diversity of exposures the overall effect of which are pending on the certain case. Chemicals in general have a major impact on human and ecosystem health and highlighting the increasing need for effective and integrated means of risk assessment and exposure evaluation in human populations and biological ecosystems is crucial. This is not a trivial task and requires not only biomonitoring and exposure assessment but also combination of risk assessment with regulatory measures and actions. Harmonization in study methodologies by implementing OECD's adverse outcome pathway (AOP) approach and systematic dealing with confounders is required for a better characterization of exposure and understanding of the effects.

WHAT PROPERTIES ARE REQUIRED FOR NANOPREPARATIONS TO BECOME EFFECTIVE ANTICANCER MEDICINEDS?

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Tumor therapy, especially in the case of multidrug resistant cancers, could be significantly enhanced by using siRNA down-regulating the production of proteins, which are involved in cancer cell resistance, such as Pgp or survivin. Even better response could be achieved is such siRNA could be delivered to tumors together with chemotherapeutic agent. This task is complicated by low stability of siRNA in biological surrounding. Thus, the delivery system should simultaneously protect siRNA from degradation. Additionally, these nanopreparations (first of all, lipid-based, such as liposomes and lipid-core micelles) can be loaded into their lipidic compartment with poorly water soluble chemotherapeutic agents, such as paclitaxel. In experiments with cancer cell monolayers, cancer cell 3D spheroids, and in animals with implanted tumors, it was shown that such co-loaded preparations can significantly down-regulate target protein components of cancer cell defense mechanisms, enhance drug activity, and reverse multidrug resistance.

In order to specifically unload such nanopreparations inside tumors or even at different cancer cell compartments, we made them sensitive to local tumor-specific stimuli, such as lowered pH, hypoxia, or overexpressed certain enzymes, such as matrix metalloproteases. Using pH-, hypoxia-, or MMP2-sensitive bonds between different components of nanopreparations co-loaded with siRNA and drugs, we were able to make the systems specifically delivering biologically active agents in required sites in tumors, which resulted in significantly improved therapeutic response.

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BIOMEDICAL APPLICATION OF NANO-SIZED POLYION COMPLEXES

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The primary focus of this lecture will be the discussion of the chemistry, characterisation and applications of nanoscale size polyion complexes formed by ionic block copolymers and polypeptides for the delivery of these polypetides to the body. Examples include delivery of antioxidant enzymes (e.g. superoxide dismutase, catalase), stoichiometric and catalytic scavengers of organophosphorous toxins (butirylcholine esterase, organophosphate hydrolase) and neurotrophins (brain-derived neurotrophic factor, glial cell line-derived neurotrophic factor). The applications include treatments of obesity, stroke, Parkinson's disease, RETT syndrome, organophosphorous toxins poisoning, and some others medical conditions that have been demonstrated using animal models.

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NANOCOMPLEX OF CYTOCHROME C WITH CARDIOLIPIN: STRUCTURE, CATALYTIC PROPERTIS, AND ANTICANCER PERSPECTIVES

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Complex of cytochrome c with a mitochondrial phospholipid cardiolipin (CytCL) plays the crucial role in apoptosis initiation in mammalian cells, including cancer cells. We investigated the structure of the complex using small angle X-ray scattering measurements, dynamic light scattering, UV-spectroscopy, spectrofluorometry and, thermolens spectroscopy. CytCL nanoparticles are small spheres build of conformationally modified CytC (molten globe) covered by the cardiolipin monolayer. They have the hydrophobic surface and tend to aggregate in water solution in concentrations above several dozen microM. Experiments with Langmuir monolayers of CL, CytC, and CytCL showed that on the hydrophobic surface CytC stratified with polar groups on the globule surface turned to water phase and non-polar – to the hydrophobic media. The mechanism of the complex penetration through the biological membranes was proposed.

CytCL nanospheres were shown to catalyze two reactions producing lipid free radicals both in aqueous and hydrophobic solvents. The first is the lipid hydroperoxide decomposition and the second – polyunsaturated fatty acid peroxidation in the presence of H_2O_2. The mechanism of the reactions was investigated by measuring the kinetics of chemiluminescence, associated with free radical reactions. These reactions proceed not only in CytCL solutions in water or chloroform, but also in suspensions of mitochondria. In collaboration with C. Sarisozen, N. Filipczak, and V. Torchilin, we have discovered that CytCL induce apoptosis and kill cancer cells in culture, both sensitive and resistant to anticancer drugs. The idea of the research appeared during discussion at Bionanotox 2016.

LIPOFUSCIN GRANULES OF THE EYE RETINAL PIGMENT EPITHELIUM: SOURCE OF THE RADICALS AND AUTOFLUORESCENCE

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Lipofuscin granules (LGs) accumulate in the cells of retinal pigment epithelium (RPE) with age, particularly in patients with hereditary diseases. These granules are heterogeneous, being composed of mixtures of proteins and lipids, including more than 21 different fluorescent compounds that are bisretinoids and their photo-oxidation and photo-degradation products. These fluorescent compounds are situated in the interior of LGs [1, 2].

Bisretinoids represent the main source of LGs fluorescence and exhibit phototoxic properties. Photosensitization of LGs with blue light can generate reactive oxygen species [3]. Recently we have shown that age-related decrease of melanin concentration in the human RPE cells is a result of melanosomes fusion with lipofuscin granules and then melanin destruction by superoxide radicals light-induced generated by these granules [4].

Regarding the LGs fluorescence, we have shown the defined differences in fluorescence properties and fluorescence kinetics between chloroform extracts obtained from cadaver human eyes with and without signs of pathology [5-7]. These differences hold promise for the future development of fundus autofluorescence imaging

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A CONCEPT OF CONSTRUCTING NEW-GENERATION HYBRID BIOTECHNOLOGICAL WOUND DRESSINGS

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To optimize healing of skin defects and diseases using novel technologies and materials is an important issue, which attracts attention of researchers in fundamental science and clinical practice since the number of injuries caused by burns, traumas, surgical interventions, cancer, and other diseases is growing continuously. Hundreds of surgical and therapeutic devices are used to cover and repair skin defects, and various materials and drugs are used to fabricate them. Principles and methods of treating skin wounds are determined by various factors: the depth and severity of the injury, the wound healing phase, the wound site, the degree of wound contamination, the patient's concomitant diseases, and the drugs taken by the patient. The main principle of wound treatment is debridement and creation of optimal conditions for healing. The study discusses a new approach to constructing hybrid biotechnological wound dressings based on nonwoven membranes prepared by electrospinning from resorbable elastomeric copolymers of beta/gamma-hydroxybutyrate and bacterial cellulose films combined with drugs and epidermal cells differentiated from the adipose tissue MSCs. The constructed biotechnological wound dressings were successfully tested in experiments with laboratory animals with model skin wounds, as shown by plane geometry, histology, and molecular markers characterizing healing processes.

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BENEFIT-RISK RATIO OF INTAKE OF CANNED SAURY (*Cololabis saira*), CONTAINED ESSENTIAL FATTY ACIDS AND HEAVY METALS

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Contents of heavy metals and polyunsaturated fatty acids (PUFA) were studied in canned saury (*Cololabis saira*), which is a popular product in Russia. Canned saury is known to be a valuable source of PUFA - eicosapentaenoic acid (20:5n-3, EPA) and docosahexaenoic acid (22:6n-3, DHA). To obtain the recommended personal dose of EPA+DHA of 1 g per day for prevention of cardiovascular and neural diseases, one needs to consume 26 - 76 g of canned saury of studied popular brands. Analysis of heavy metals demonstrated that lead content in one brand and cadmium content in most of studied samples exceeded the European Commission standards for food fish. However, values of benefit-risk ratio of fish intake, quantified as hazard quotients, showed that canned saury is safe and valuable product for human nutrition.

APPLICATION OF ECOLOGICALLY FRIENDLY POLYSACCHARIDE – GELLAN FOR ENHANCED OIL RECOVERY

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The results of laboratory and oilfield tests on the applicability of ecologically friendly polysaccharide – gellan for permeability modification treatments of oil bearing heterogeneous strata for restriction of water production and increase of oil flow rate are presented. Filtration experiments conducted on sand packs reveal that the ability of aqueous gellan solution to transform to gel upon contacting with reservoir brine can be exploited for both near wellbore and in-depth plugging of watered out filtration channels. Sand pack flooding experiments showed the excellent plugging ability of gellan with respect to high-conductive filtration paths and redirection of injected water flow inside of water flooded oil reservoirs. According to pilot test results, the amount of incremental oil produced from 5 production wells during 19 months was equal to 15 000 tons. It was demonstrated that polysaccharide – gellan is more effective to decrease the permeability of high-conductive watered out filtration channels in comparison with poly(acrylamide) or hydrolyzed poly(acrylamide) that are traditionally used for enhanced oil recovery.

"INDIVIDUAL PROFILING": NANOPARTICLE'S BLOOD-BRAIN BARRIER PASSAGE AND TISSUE DISTRIBUTION CHARACTERISED BY IN VIVO IMAGING

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Objectives The vessels in the mammalian brain have a very tight lining – the so called blood-brain barrier (BBB) - and therefore objects in the nanometre range cannot passively diffuse through this barrier. However, although highly regulated, the BBB also allows exchange of substances by transport mechanisms and via such mechanisms also rather large objects such as nanoparticles (NPs) may enter into brain tissue. This possibility may be an advantage for drug transport but it may be also a disadvantage with regards to environmental toxicology. In any case, to understand the BBB passage of NPs is of utmost importance. Here we visualised with in vivo imaging of the retina - which is brain tissue - how different groups of NPs interact with the BBB and how modifications of the production protocol leads to different outcomes.
Methods In vivo Confocal Neuroimaging (ICON) was used for real time monitoring of the bio-distribution of fluorescent NPs at the blood-retina barrier (which is virtually the same as the BBB). To this end, rats were anaesthetised and fluorescence-labeled nanoparticles were injected into the tail vein. The retina was imaged with a confocal laser scanning microscope via the eye before and after application of the NPs. A range of different polymeric NP were provided by our partners and were tested with ICON: polybutylcyanoacrylate (PBCA) NPs, poly(lactic-co-glycolic acid) (PLGA) NPs and polyvinylpyrrolidone (PVP) NPs.
Results With ICON we could distinguish 3 different kinds of distribution (i) short-lasting fluorescent signal in the vessel (minutes) (ii) long-lasting staining of the vessels (hours) (iii) transition of the fluorescent signal from vessels to parenchyma. We investigated the influence of the different polymers, surfactants, size and of the active compound and it turned out that manipulation of each single factor resulted in a significantly altered pharmacokinetic behaviour of the NPs. Data from our work and from others indicate that **in vivo a corona of** blood-proteins around the NPs may substantially alter physical properties of the nano-system, which will ultimately influence the interaction with the BBB.
Conclusions Under in vivo conditions various physiological interactions between the organism and the particle can take place, as for example, the formation of a corona from body fluids.

Therefore minor differences in the texture of the nano-systems may induce significant differences in the resulting "secondary" nano-systems and as a consequence an altered behaviour at the BBB. Therefore in vivo testing of NPs with biological systems is mandatory.

ECOTOXICOLOGICAL PROFILE OF LANTHANIDES

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The application of rare earth elements (including lanthanides) in different sectors of the world economy has significantly increased during last two decades. As an example, the battery pack in a hybrid car may contain up to 10 kg of lanthanum, and its electric motor nearly 1 kg of neodymium. In parallel to the anthropogenic disruption of biogeochemical cycle of lanthanides, the risks related to exposure of biota to the elevated concentrations of lanthanides also rise. In spite of increased risks the ecotoxicological effects of these elements and their fate in the environment are poorly understood. In the current study, the toxicity of soluble salts of lanthanides ($La(NO_3)_3 \cdot 6H_2O$, $Ce(NO_3)_3 \cdot 6H_2O$, $Pr(NO_3)_3 \cdot 6H_2O$, $Nd(NO_3)_3 \cdot 6H_2O$, $Gd(NO_3)_3 \cdot 6H_2O$) were evaluated using a test battery composed of aquatic species representing different trophic levels: freshwater microalgae, duckweed, marine bacteria, protozoa and freshwater crustaceans. Toxicity tests with salts of lanthanides revealed that (i) pollution by lanthanides may significantly disturb the equilibrium of aquatic ecosystems; (ii) bacteria and microalgae were the most sensitive to lanthanides according to the results from the used laboratory assays; (iii) although lanthanides are a chemically uniform group of elements, their behaviour and ecotoxicological profile slightly differ depending on test media.

SAFE USE OF ANTIMICROBIAL NANO-COATINGS FOR HIGH-TOUCH SURFACES IN HEALTH-CARE SETTINGS: EC COST ACTION NETWORK AMICI

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Objectives: Infections and infectious diseases are considered a major challenge to human health in healthcare units worldwide. The EC COST Action CA15114 AMiCI (2016-2020) is involved in development, regulation, and use of novel Antimicrobial coatings (AMCs) for the prevention of health-care associated infections. The AMiCI network currently comprises participants of more than 60 universities, research institutes, and companies across 29 European countries.

Methods: AMiCI consortium (75 members from 24 countries) participated in facilitated discussions (flip-chart sessions) during the kick-off meeting of AMiCI in Heerlen, Netherlands, Nov.17, 2016.

Results and discussion: Inspired by the above described flip-chart discussions, and backed up by the state-of-the-art literature data, the current presentation will guide the audience through different aspects of quality and safety aspects of AMCs use and application on frequently-touched surfaces in healthcare settings. As the most often used biocidal (nano)materials in AMCs for high-touch surfaces are nanosilver, CuO and TiO_2 that are intrinsically toxic (silver, copper) or toxic upon photoactivation (TiO_2), we will discuss on

specific harmful aspects of use of AMCs. The latter aspects are the potential induction of antimicrobial resistance (AMR) and/or ecotoxicological effects. In this context we will also address the risk-benefit aspects of these novel coatings and possibilities to minimize those risks at the level of safe-by-design.
Conclusion: Weighing the beneficial and adverse effects of AMC in healthcare settings requires the thorough assessment on the following topics using multidisciplinary and multilevel approaches:
Ecotoxicological hazard needs to be evaluated proactively, before use of AMCs in healthcare settings' surfaces in the environment of patients
The lessons learnt in AMR should be taken on board when assessing the risks of AMCs
The quality, efficacy and safety evaluation of antimicrobial materials in healthcare settings should be addressed at the level of safe-by-design approach
Involvement of concerned stakeholders in the risk-benefit analysis is important for the responsible development of AMCs
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ADVERSE EFFECT OF SILVER NANOPARTICLES TO THE MICROBIAL COMMUNITY OF WASTE-WATER TREATMENT PLANT

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The number of consumer products on the market containing nanoparticles already exceeds 1800 (www.nanotechproject.org). Silver nanoparticles (AgNPs) have the highest degree of commercialization, mainly due to their biocidal properties. Nanoparticles may release from the nano-enabled products (e.g., antimicrobial coatings) along their life-cycle and finally end up in the wastewater treatment plants (WWTP). The extent to which biocidal nanoparticles (e.g. Ag, CuO and ZnO NPs) may influence the WWTP microbial community is practically unknown.
The current presentation focuses on the toxicity assessment of AgNPs to the WWTP microbial community. Non-coated (~80 nm), polyvinylpyrrolidone (PVP, ~10 nm), protein-coated AgNPs (~10 nm, collargol) and $AgNO_3$ as an ionic control were studied. Wastewater was sampled from the Estonian municipal WWTP. Both, original (non-filtered) and filtered (0.2 µm) wastewater were analysed to evaluate the modulating effect of the suspended solids on AgNPs physical-chemical properties. Stability of AgNPs in the wastewater was assessed by the UV-vis analysis. Toxic effect of the AgNPs and Ag-ions on the wastewater microbial community was evaluated by incubating the wastewater samples with AgNPs/ions for 3 and 11 days. Viability of the microbial community was evaluated by plating and counting the colonies on Plate Count Agar and by staining the cells with the Alamar Blue. From these data IC_{50} values (mg Ag/L) were calculated. Dissolution of the AgNPs in the wastewater was quantified by AAS, and bioavailability of AgNPs by Ag-sensing recombinant luminescent bacteria.
Results showed that AgNPs were unstable in the wastewater and Ag-ions recovery was ~0.1%. The IC_{50} values of the studied Ag-compounds ranged from 15-2700 mg Ag/L and the order of the toxicity was as follows: $AgNO_3$>PVP-AgNPs>collargol>non-coated AgNPs. AgNPs dissolution study revealed that the toxicity of AgNPs to the microbial community of WWTP was caused by the released ions.
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BIOCOMPATIBLE FILMS AND POROUS SCAFFOLDS BASED ON CHITOSAN CROSS-LINKED WITH GENIPIN OR GLUTARALDEHYDE

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The conflicting data on the toxicity of reaction products of chitosan and glutaraldehyde (GA) may result from the use of samples with a high content of crosslinking agent (30 and 70% of chitosan amino groups were substituted) in a number of studies [1,2]. The influence of the conditions of the formation of chitosan hydrogels crosslinked with GA or genipin (the polysaccharide molecular weight, pH level, and concentration of the chitosan solution) on the gel time and the properties of biopolymer films and porous scaffolds for tissue engineering was studied. The resulting scaffolds had different structures (morphology, degree of anisotropy, average pore size) and moisture-retaining capacities.

The cytotoxicity of biodegradable scaffolds based on chitosan with a low content of genipin and GA was studied for the first time. It was shown that water-insoluble biopolymer scaffold based on chitosan hydrogel with MMs of 320 and 190 kDa crosslinked with GA and genipin (0.005 and 0.01 mol/mol of amino groups of chitosan) are biocompatible. By confocal laser microscopy it was demonstrated that cells were uniformly distributed in all of the scaffold samples and actively grew and proliferated during in vitro cultivation.

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APPLICATION OF ENZYMES FOR THE PREPARATION OF BIOLOGICALLY ACTIVE NUCLEOSIDES

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Enzymes are widely used in industry for carrying out various transformations and producing useful substances and materials. An enzymatic transglycosylation reactions, a transfer of the carbohydrate residue from one heterocyclic base to another, is being actively developed and applied for the synthesis of practically important nucleosides. These reactions are catalysed by nucleoside phosphorylases (NPs) that perform reversible phosphorolysis of nucleoside to yield the corresponding heterocyclic base and 1-phosphate of monosaccharide.

Nuc + pi ↔ Sug-p + Base

The equilibriums of these reactions are shifted towards formation of nucleosides, more significantly in the case of purine nucleoside and may be considered as a driving force for the total transglycosylation process. NPs are used in industry for the synthesis of drugs (Cladribin, Fludarabine and Nelarabin) and practically important nucleosides. The ability of uridine phosphorylase (UP) and purine nucleoside phosphorylase (PNP) to cleave 2'-deoxynucleosides, ß-D-arabinofuranosylnucleosides and to catalyse their formation is widely used. In these cases, enzymatic methods for creating of the N-glycosidic bond can compete with the chemical synthesis.

During the course of our work the following key results were obtained.

1. A large library of modified nucleosides synthesized at IMB RAS was used to study the substrate specificity of E. coli UP and PNP. We measured the kinetic parameters and equilibrium constants for the reaction of enzymatic phosphorolysis of about 60 derivatives of uridine and adenosine, modified at different positions of the heterocyclic base and at the 2'-, 3'- and 5'-positions of ribose residue.

2. The combined kinetic and structural data provide clear evidence that UP binds uridine in the most energetically unfavourable conformation, which, to the best of our knowledge, has no precedents in the enzymes of nucleic acid metabolism. We have identified the similarities of the architecture of active sites of UP and PNP when bound to the substrate in a tense

conformation, in which the heterocyclic base is located close to 2'-proton of the carbohydrate moiety.
3. We have experimentally shown that the outcome of transglycosylation is governed entirely by equilibrium phosphorolysis constants of initial and final nucleosides.
This work was supported by the Russian Science Foundation (grant No. 16-14-00178).

NATURAL NANOSTRUCTURES (LIPOPROTEIDS OF BLOOD PLASMA) PLAY THE IMPORTANT ROLE IN THE DEVELOPMENT OF PATHOLOGICAL STATES

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Low density lipoproteins (LDL) of blood plasma are natural lipid-transporting nanoparticles. LDL peroxidation leads to the accumulation of primary (hydroperoxides) and secondary (dicarbonyls) products. This is accompanied by a modification of the LDL particles structure , as a result of which they acquire the ability to be captured by scavenger receptors of cells and induce of atherogenic damage in vessel wall during cardiovascular diseases and in diabetes. It has been shown that the co-oxidation of LDL lipids and glucose causes intensification of free radical processes and the glucose oxidation product - methylglyoxal produces a superoxide radical anion in the process of interaction with amine compounds. It was established that in patients with diabetes mellitus the oxidation of LDL is significantly higher than in patients with atherosclerosis. Nevertheless the particles of more atherogenic lipoproteins (a) are subject to free radical oxidation to a much lesser degree than LDL particles, but become susceptible to oxidation after modification with low molecular weight dicarbonyls. LDL modified with glyoxal and methylglyoxal are quite slowly eliminated from the blood flow of primates (rhesus monkey), whereas malonyldialdehyde-modified LDL disappear from the bloodstream very quickly.
The work was carried out with the partial financial support of the grant of the Russian Science Foundation No. 14-15-00245.
Main publications: Lankin V. et al., in: Handbook of Lipoprotein Res. 2010, Nova Sci.Pub., 85-107; Lankin VZ et al., Mol Cell Biochem 2014, 395(1-2): 241-252; Lankin VZ et al., J.Diabetes 2015, 8(3): 398-404; Lankin VZ, Tikhaze AK. Curr Aging Sci. 2017, 10(1): 18-25.

GENE AND CELL APPROACHES IN REGENERATIVE MEDICINE

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Regenerative medicine is a modern field of biomedicine that operates a number of nature-granted or synthetic approaches to induce tissue repair and recapitulation of normal function lost due to disease or injury. In this report we shall summarize our effort in the field and will discuss results in experimental application of gene and cell therapies.
We have developed a number of methods to induce angiogenic response in ischemic tissue focusing on non-viral delivery of human growth factor genes (VEGF165, HGF, angiopoietin-1) directly to ischemic tissues. To achieve higher efficacy and improve performance we also applied combined delivery of these genes and found them to have superior efficacy compared to each gene alone.
Another method we use is application of cell therapies with a focus on adult mesenchymal stem cells (MSC) isolated from adipose tissue. MSC is widely used for therapeutic application yet we decided to address the problem of their delivery and switched from injection to cell sheet engineering to increase cell viability after implantation. We found cell sheets from MSC to be more efficient that injected suspension in a model of limb ischemia inducing higher perfusion and angiogenic response in skeletal muscle of experimental mice.
Furthermore, we set to use viral delivery of growth factor genes to increase the MSC therapeutic potential via paracrine function. In our studies gene-modified MSC showed better performance in terms of tissue perfusion and protection compared to mock-treated or non-transduced cells.
Thus, summarizing our experience we may conclude we possess a vast arsenal of methods not limited to gene or cell therapies, but also including their combination and tissue engineering

methods. Attractive options for further development include application of cell-free materials from stem cell secretome, extracellular vesicles or matrix preparation known to possess regenerative and healing potential.

EXTREMELY LOW MAGNETIC FIELD AS A PERSPECTIVE ALTERNATIVE FOR MEMBRANE MICROVISCOSITY REGULATION

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Lipid membrane vesicles e.g. liposomes, exosomes and hybrid lipid nanoparticles are almost ideal carriers for delivery of intelligent drugs and genes. Nowadays one of the key problems in clinical application of this type of drug delivery system is the controlled loading and release of cargo. The typical protocol of drug loading usually includes sonication for a few minutes, however now a number of disadvantages of this method are disclosed. Prolonged sonication usually causes membrane damage especially in case of natural vesicles (exosomes) because of overheating and lipid oxidation. Here we suggest an alternative way for membrane loosening; the main idea if that is the application of extremely low magnetic field in pair with complex formation with magnetic nanorods.
We have compared both ways on the example of anionic liposomes labeled with BODIPY-modified DPPC sensitive to changes of membrane microfluidity. Here an analytical signal is polarization of fluorescence and this value is as higher as higher is membrane rigidity.
We have compared values of fluorescence polarization for both protocols. Sonication was conducted in ice to prevent early overheating, however 30 minutes of exposure lead to membrane destroy. Even 5 minutes of sonication provide a significant disturbance of membrane with value of fluorescence polarization typical for damaged liposomes (found in separate experiment). On the other hand complex of anionic liposomes with magnetic nanorods has demonstrated smooth membrane loosening more attractive for further application for biomedical goals.
Data of mathematical modeling shows that rotational-vibrational motions of f-MNPs bounded to liposomes mainly cause probable perturbation of membrane. The nanorods bounded to membrane surface are set into a complex oscillating motion similar to motion of a double-bladed kayak paddle.
This work is supported by RSF 14-13-00731

INVESTIGATION OF ANTISEPTICS' ANTIMICROBIAL ACTIVITY AGAINST STAPHYLOCOCCUS EPIDERMIDIS

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The researches on assessment of antiseptics' antimicrobial activity (widely known and used in clinical practice) in presence of nanodisperse cerium dioxide for the purposes of definition the possibility of dressings' elaboration for treatment of surgical wounds were carried out.
Key words: nanodispersed cerium dioxide (NCD), antimicrobial activity, antiseptic, Lavasept, Chlorhexidine, Dioksidin, Miramistin, cationic surfactants, *Staphylococcus epidermidis*.
Nanocrystalline cerium dioxide (NCD) is a promising material for biomedical applications [1, 2]. Advantages of NCD application are determined by two main factors: high oxygen non-stoichiometry and low toxicity. The first factor determines participation of NCD in redox processes of living cells, particularly under oxidative stress conditions. The second factor provides comparative safety use of cerium dioxide nanoparticles *in vivo*. Regenerative ability of oxygen non-stoichiometry is specific propertiy of NCD. It means that NCD nanoparticles after participation in redox processes in a relatively short period of time are able to return into its original state, which ensures prolonged action *in situ.*

Thereby the study of NCDs'influence on antiseptics'antimicrobial activity is important with the purposes of elaboration of integrated medical devices.
The objects of study were nanodispersed cerium dioxide (NCD) solutions, which were stabilized by ammonium citrate. The other part of nanodispersed cerium dioxide solutions were without additional stabilizers. Series of antiseptics were presented by the following preparations (commercially available) with concentrations widely used in medical practice: Lavasept (0.2%); Chlorhexidine (0.02%); Dioksidin (1.0%); Miramistin (0.01%); and cationic surfactants, quaternary ammonium compounds. Preparations for studies were prepared in ratios of antiseptic: NCD (stabilized and unstabilized) equal to 1:1 and 1:2, respectively.
The results showed that, in the cases of stabilized and unstabilized NCD, antiseptic' concentration practically didn't change its antimicrobial activity against *Staphylococcus epidermidis*.
Test cultures'zones of growth retardation practically were the same at starting concentrations of antiseptics, and Lavasept, Chlorhexidine, Miramistine dilution in 2 times, wherein sensitivity index was satisfactory - the growth retardation was about 15 - 25 mm.
Stable preservation and even a slight increase of antiseptics'antimicrobial activity were observed in presence of unstabilized and stabilized NCD. These antiseptics contained a labile chlorine atoms in its structure - polymeric quaternary ammonium halides.
Stabilized sol of NCD is characterized by a negative value of z-potential (- 21 mV), unstabilized sol of NCD has a positive value of z-potential (appr. + 30 mV). Negative surface charge of sol can contribute to better adsorption on surface of nanoparticles of biologically active organic cations of quaternary ammonium compounds releasing active chlorine ions. However, in the system unstabilized NCD:halides increase of antimicrobial activity was also observed, which allowed to make a conclusion about symbiotic activity of mixture NCD:quaternary ammonium polymer halides regardless of medium acidity, particle size of cerium dioxide, methods for preparing cerium initial solutions.
Thus, the solutions of nanodispersed cerium dioxide can be combined with any antiseptics without suppression of their antimicrobial activity.

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PRODUCTION AND PROPERTIES OF BACTERIAL CELLULOSE BY THE NEW STRAIN OF ACETIC ACID BACTERIA *KOMAGATAEIBACTER XYLINUS* B-12068

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To increase the scale of production of bacterial cellulose and to widen the range of its applications, it is necessary to have bacterial strains capable of synthesizing high yields of this valuable product of biotechnology. Therefore, much of recent research has been focused on finding new cellulose-producing strains and improving the fermentation techniques. The strain of acetic acid bacteria, *Komagataeibacter xylinus* B-12068 – a producer of bacterial cellulose (BC) has been isolated and characterized. The effects of cultivation conditions (carbon sources, temperature, and pH) on BC production and properties were studied in surface and submerged cultures. Glucose was found to be the best substrate for BC production among the sugars tested; ethanol concentration of 3% (w/v) enhanced the productivity of BC. The highest BC yield (up to 17.0 g/l) was obtained under surface static cultivation conditions, in the modified Hestrin-Schramm medium supplemented with ethanol, at pH 3.9, after 7 days of cultivation in the thinnest layer of the medium. Elemental analysis, emission spectrometry, scanning electron microscopy, differential thermal analysis, and X-Ray were used to investigate the structure and physical properties of the BC produced under different conditions. Based on *in vitro* cell culture investigations the native cellulose membrane did not

cause cytotoxicity upon direct contact with mouse fibroblast cells and was highly biocompatible

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NEW IMMUNOASSAYS FOR DETECTION OF ORGANIC COMPOUNDS

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The immunochemical methods based on the specific and high affinity antibodies are widely used for high-throughput screening (HTS) and monitoring of organic compounds. At first it is important for detection of poisons and toxic organic chemicals like: pesticides, detergents, endocrine disrupters (EDC), dyes, mycotoxins, explosive and etc. The Enzyme-Linked Immunosorbent Assay (ELISA) is one of widely used immunoassays. Moreover, ELISAs are modified to make its more simple, quick and cheap without any instruments or with using available device like smart phone. Recent trend for development and application of new immunochemical methods will be given.

More details could be found in our recent publications and reviews.

INACTIVATION OF HEPARIN BY QUATERNIZED DERIVATIVES OF CHITOSAN

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Low molecular weight (LMWH) and unfractionated heparins (UFH) are widely used in surgical operations, prevention of thrombosis in venous thromboembolism, acute coronary syndrome, extracorporeal circulation and hemodialysis. Therapy with heparin is associated with a number of complications in the first place is the occurrence of bleeding. To neutralize the activity of the heparins used a specific antagonist – protamine sulfate (PS). However, it causes numerous side effects, from mild hypotension to cardiac arrest. Therefore, the search for new antidotes heparins is an actual problem.

For this purpose were synthesized quaternized derivatives of low molecular weight chitosan with different substitution degrees of 9, 40 and 98 % (QCh9, QCh40, QCh98 respectively). The structure of the obtained derivatives was defined by IR-spectroscopy and proton magnetic resonance. Chitosan derivatives were characterized with positive zeta-potential (33–51 mV) and solubility from 2 to 100 mg/mL in pH 7.4 and 25°C.

In recent years, the advantages of low molecular weight heparin preparations over unfractionated preparations. Therefore, the paper presents data for LMWH. To determine the procoagulant effect of the studied quaternized chitosan derivatives, their influence on the time of fibrin clot formation in human plasma containing LMWH was analyzed. QCh98 in concentrations of 0.0014–0.0029 mg/mL, as well as PS, partially decreased the $TC_{ReaClot}$ of plasma with LMWH (1.56 aXa U/mL); the observed decrease was more effective than the neutralization of aXa activity of LMWH by PS. It is worth noting that such high doses of heparin are used in extracorporeal circulation only.

The effect of the concentrations of the derivatives used in the neutralization experiments on anticoagulant activity on blood cells was studied. PS and QCh98 equally practically no effect on hemolysis. Incubation of platelet rich plasma containing 0.0029 mg/mL QCh98 (without subsequent additions of adenosine diphosphate), did not lead to platelet aggregation. Adding to the plasma QCh98 in high concentrations, enhanced aggregation of platelets that can be applied to compounds or materials with hemostatic activity.

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POLYSACCHARIDE MICROCONTAINERS WITH A FUNCTIONALIZED SURFACE FOR DELIVERY OF LIPOPHILIC ANTICANCER DRUGS: PREPARATION AND *IN VITRO* STUDY

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Many antitumor drugs are lipophilic which significantly complicates their use. Therefore development of new carriers to deliver hydrophobic drugs is a challenge of biomedicine. Recently, a novel technique to prepare polysaccharide microcontainers (MCs) using low-frequency ultrasound was developed by Borodina et al [1]. By this technique, a lipophilic drug was efficiently encapsulated in MCs with core-shell structure by us [2]. The soybean core of MCs was loaded with the drug, while the shell was based on chitosan and xanthan gum. However, since chitosan has not been approved by FDA until now, we decided to replace it with DEAE-dextran.

The aim of this study was to obtain MCs with a functionalized surface and to study them *in vitro*. MCs with mean diameter of 350±35 nm were fabricated from DEAE-dextran (150 kDa) and xanthan gum (20-300 cP) by low-frequency ultrasonication. MCs were coated with poly-L-lysine (PLL, 40-60 kDa) or polyethylene glycol (PEG, 1,5 kDa). Modification with PLL allowed to obtain a positively charged MCs-PLL (+25 mV compared to -40 mV of non-coated MCs). To prevent MCs capture by reticuloendothelial system, the stealth MCs were prepared using PEG coating. The natural antitumor drug thymoquinone (TQ, 100 mg/ml) or Nile Red dye (10 mg/ml) was encapsulated in MCs. Cytotoxicity was determined by MTT-test using human breast adenocarcinoma MCF-7 cells. Blank MCs (without TQ) were not cytotoxic, while MCs with TQ exhibited IC50 values which markedly differed. The highest cytotoxicity value after 24 h incubation was found for MCs-PLL (IC50 0,095 mg/ml), while MCs-PEG showed minimal IC50 (0,5 mg/ml). The accumulation of MCs within the cells was studied by flow cytometry and confocal laser microscopy. Penetration and accumulation of the initial MCs, MCs-PLL and MCs-PEG samples differed. Thus, within first 30 min MCs and MCs-PLL were found to accumulate within the cytoplasm, while MCs–PEG were localized on the cells membrane. The polymer shell of MCs was also functionalized with magnetite nanoparticles, in order to study drug release induced by non-heating magnetic field in the future.

Thus, the existing technique was optimized, and a simple proposed approach allowed to carry out additional MCs modifications. Functionalization of the MCs surface with PLL or PEG resulted in increase of MCs stability, surface charge optimization and could provide development of a targeted drug delivery system.

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RECOMBINANT SUPEROXIDE DISMUTASE 1 INCORPORATED INTO POLYMERIC NANOPARTICLES FOR OPHTHALMIC APPLICATION

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Inflammatory eye diseases are the most prevalent eye pathologies which lead to partial disability and, sometimes, to the complete loss of vision. Among these diseases, the most severe one is uveitis, inflammation of uveal tract involving both outer and inner structures of the eye. The incidence of uveitis in the structure of common ocular pathology is 5-12%, this disease being responsible for 7–20% of blindness.

One of the approaches to the treatment of inflammatory processes at uveitis is the use of antioxidants, in particular, superoxide dismutase1 (SOD1). However, only 5–10% of applied drug during topical instillations penetrates the cornea and reaches the intraocular tissues.

In order to enhance the efficacy of enzyme penetration through the cornea barrier, SOD1 containing polymeric nanoparticles was prepared. As a result, nanoformulations based on complexes of recombinant human enzyme SOD1 with methoxy-poly(ethyleneglycol)-poly(L-lysine)$_{50}$ block copolymer cross-linked by 3,3'-dithiobis(sulfosuccinimidylpropionate), as well as nanoformulations based on double layer complexes of SOD1 with protamine and block copolymer of methoxy-poly(ethylene glycol)-block-poly(L-glutamic acid sodium salt) cross-linked with glutaraldehyde were obtained.

In an experimental model of immunogenic uveitis in rabbits, the biochemical indicators of the disease were significantly improved during topical instillations of the solution of a recombinant SOD1 incorporated into polymeric nanoparticles compared to the same treatment by an aqueous solution of the enzyme or placebo.

Significant reduction of the duration of corneal and eyelid edema, decrease in the amount of fibrin clots, mitigation of lens opacity, as well as mitigation of conjunctival hyperemia, increase in antioxidant activity in the lacrimal fluid, and decrease in leukocyte count, the total protein and α_2-macroglobulin activity in the aqueous humor of rabbits were observed. The data have been confirmed by histological studies of various eye tissues.

The results obtained demonstrate the potential of nanoformulations based on SOD1 as promising therapeutic agents for ophthalmology.

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DEVELOPMENT OF LIPOSOMES FOR TARGETED CISPLATIN DELIVERY TO GLIOMA CELLS

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Brain tumors are characterized by high degree of vascularization, increased vascular permeability and impairment of the blood-brain barrier integrity. These features of the vasculature structure in the brain tumor allow nanoscale particles, such as liposomes, to penetrate from the bloodstream and accumulate in the tumor tissue. Vascular endothelial growth factor (VEGF) and its receptor type II (VEGFR2) are promising targets due to their overexpression by not only core tumor cells but also by migrated glioma cells, which are responsible for resistance and rapid progression of brain tumors.

The goal of this work was to develop the stable cisplatin-loaded liposomes with high binding affinity to glioma cells.

The synthesis of liposomes was carried out by emulsification of lipid film consisting from the mixture of different phospholipids in an aqueous solution of platinum salt. Conjugation of liposomes with monoclonal antibodies to VEGF and VEGFR2 was performed by attaching activated antibodies to liposomes containing outer maleimide groups. Physicochemical characteristics of liposomes were studied by dynamic light scattering, transmission electron microscopy and X-ray fluorescence analysis. The cytotoxicity of obtained formulations was studied on glioma C6 cells using MTT-test. The binding affinity was analyzed on fixed and live glioma cells (C6, U-87 MG) by confocal microscopy. The study of pharmacokinetics was carried out by determination of Pt concentration using ICP MS during 48 hours after intravenous administration of formulations.

The developed liposomes revealed sustained drug release profile, high affinity to antigens, and increased uptake by glioma C6 and U-87 MG cells. The maximum loading capacity of liposomes was 24±3 % that exceeded twice the loading of commercially available liposomal nanoparticles (Lipoplatin®). Specific monoclonal antibodies doubled the cytotoxicity of the liposomes compared to non-targeted liposomes in VEGF-positive glioma C6 cells. Pharmacokinetic study on glioma C6-bearing rats revealed prolonged blood circulation time of the liposomes.

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MAGNETIC NANOPARTICLES ACTUATED BY NON-HEATING LOW FREQUENCY MAGNETIC FIELD AS THE MULTIMODAL TOOLS FOR THE NEW-GENERATION NANOMEDICINE

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This survey involves one of the most promising branches of new-generation biomedicine, namely magnetic nanotheranostics that uses remote control over functionalized magnetic nanoparticles (f-MNPs) by means of alternating magnetic fields (AMFs). The review is mainly focused on new approach which utilizes non-heating low frequency magnetic fields (LFMFs) for nanomechanical actuation of f-MNPs as distinguished from such traditional ones as magnetic resonance imaging (MRI) and radio-frequency (RF) magnetic hyperthermia (MH) utilizing high frequency heating AMF. Basic innovative principles and models employing various types of f-MNPs are discussed along with f-MNP shape and immobilization effects on potential nanomechanical actuation applications and LFMF and f-MNPs parameters optimization. Nanomechanical actuation approach is shown to be capable of controlling catalytic reaction rate, drug release acceleration and cell structures stimulation. It opens new opportunities in cancer therapy, targeted drug delivery, tissue engineering and regenerative medicine. It is shown that specifically designed high gradient, steady magnetic field enables diagnostic and therapeutic LFMF impact localization as well as f-MNPs biodistribution monitoring in the deep tissues within the area ranging from a millimeter to a few centimeters and 3D scanning of affected region, if necessary. Proposed and already patented methods form a basis for new technological platform of new generation low-frequency magnetic theranostics which is more versatile, effective and safe than radio-frequency ones.

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STYRENE-BUTYL ACRYLATE COPOLYMERS AS NOVEL PRESSURE SENSITIVE ADHESIVES FOR SKIN APPLICATION

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The effect of the macromolecule chain microstructure (block, random and gradient) on surface characteristics and adhesion performance was investigated for novel copolymers of styrene (S) and butyl acrylate (BA) with different content of co-monomers. All copolymers were synthesized by RAFT-polymerization, which provided the given sequence of monomer links within macromolecule as well as composition homogeneity and molecular weight.
It was found that gradient and block copolymers demonstrated micro-phase separation even for styrene concentration lower than 60 mol. %, so that "matrix (BA) – inclusion (S)" structure was formed. Energy characteristics of films on the air, polar and nonpolar liquid interfaces were determined by the contact angle technique. Films were prepared by casting solution from toluene and acetone.
It was found that gradient and block copolymers with styrene content within 30-50 mol. % diapason can be used as a promising pressure sensitive adhesives. The strength of adhesive joints with skin and model substrates was estimated using 180 degree peel test.
It was suggested a new methodological approach to study the effect of chain micro structure and solvent nature on adhesive performance of binary copolymers. The approach is based on a complex analysis of the magnitude of thermodynamic work of adhesion in model systems "polymer – liquid" in combination with the results of mechanical testing, IR-Fourier spectroscopy (ATR) and quantum-chemical calculations. On the example of styrene – butyl acrylate gradient copolymer films (with 30 mol.% styrene concentration) that were formed from two different solvents, the adhesive properties were predicted and then experimentally confirmed.
Thus, the obtained results indicate that there is the fundamental possibility of control of surface characteristics and adhesive performance for binary copolymers of styrene and butyl acrylate due to variation of chain microstructure, thermodynamic nature of solvent and monomer concentration.

EXOSOMES AS NATURAL NANOSIZED CARRIERS FOR DRUG DELIVERY

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Exosomes are naturally occurring nanosized vesicles that have attracted considerable attention as drug delivery vehicles in the past few years. Exosomes are comprised of natural lipid bilayers with the abundance of adhesive proteins that readily interact with cellular membranes. We posit that exosomes secreted by monocytes and macrophages can provide an unprecedented opportunity to avoid entrapment in mononuclear phagocytes (as a part of the host immune system), and at the same time enhance delivery of incorporated drugs to target cells ultimately increasing drug therapeutic efficacy. In light of this, we developed a new exosomal-based delivery system for a potent antioxidant, catalase, to treat Parkinson's disease (PD). Catalase was loaded into exosomes *ex vivo* using different methods: the incubation at room temperature, permeabilization with saponin, freeze-thaw cycles, sonication, or extrusion. The size of the obtained catalase-loaded exosomes (exoCAT) was in the range of 100 - 200 nm. A reformation of exosomes upon sonication and extrusion, or permeabilization with saponin resulted in high loading efficiency, sustained release, and catalase preservation against proteases degradation. Exosomes were readily taken up by neuronal cells *in vitro*. ExoCAT provided significant neuroprotective effects in *in vitro* and *in vivo* models of PD. Overall, exosome-based catalase formulations have a potential to be a versatile strategy to treat inflammatory and neurodegenerative disorders.
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P01

FLUORESCENT PROPERTIES OF THE DIL-LABELED PLGA NANOPARTICLES

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DiI (1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate) is a carbocyanine dye widely used in cell biology due to its low toxicity and the tendency to give highly stable cell labeling. This dye labels cell membranes by inserting its two long (C18 carbon) hydrocarbon chains into the lipid bilayers.

In the present study, DiI was used for fluorescent labeling of the poly(lactide-co-glycolide) (PLGA) nanoparticles (NPs). The objective of the study was to evaluate the fluorescent properties of the PLGA nanoparticles with encapsulated DiI and to determine its optimal content.

The PLGA NPs were prepared by a high pressure homogenization – solvent evaporation technique. The dye-to-polymer ratios were in the range of 0.4 to 25 μg/mg. Bis(2-ethylhexyl) sulfosuccinate sodium salt (AOT) was added for better DiI encapsulation. Particle size and zeta potential were measured using a Zetasizer (Malvern Instruments, U.K.). The surface morphology and the size of the particles were confirmed by scanning electron microscopy (microscope JSM-6510 LV, Jeol Ltd., Peabody, MA). Amount of PLGA in the preparation was assessed using capillary electrophoresis (Kapel-105M, Lumex, RF); DiI assay was performed by spectrophotometry (UV-1800, SHIMADZU, Japan) and spectrofluorimetry (RF-6000, Shimadzu, Japan). Fluorescence brightness was calculated as: $brightness = \varepsilon \cdot \varphi \cdot \mathrm{N}$, where φ – quantum yield, ε – molar extinction coefficient, N – number of dye molecules per 1 nanoparticle.

Results. The mean particle sizes measured by DLS were ~100 nm with PDI of 0.097±0.030; zeta-potential was -37.38±2.28 mV. SEM showed spherical form of the NPs and sizes in the range of 67 – 141 nm.

The results of the study revealed the non-linear dependence of the brightness on the dye content in the NPs. The maximal brightness (≥ $2 \cdot 10^5$ $M^{-1} \cdot cm^{-1}$) was observed for the NPs containing 1.16–6.25 μg/mg. The NPs containing 2.18 μg/mg of DiI were the brightest and had the highest quantum yield (16.49±0.65%, $\lambda_{ex.}$= 500.nm). In the presence of AOT, the DiI loading in the NPs increased from 59 to 84%; however the quantum yields of these NPs decreased from 16% to 6%.

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P02

COMPARISON OF QUANTUM YIELDS OF Cy5.5-LABELED PLGA NANOPARTICLES PREPARED BY VARIOUS TECHNIQUES

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Fluorescent nanoparticles are of great importance in modern pharmacological studies. They are extensively used in the biodistribution studies as well as in the cell culture experiments. For successful fluorescent labeling of the nanoparticle a dye must possess certain properties such as: brightness, large Stokes shifts, resistance to photobleaching and high quantum yield, as well as their excitation and emission maxima should be within the near-infrared transparency window.
In the present work, we compared relative quantum yields of the poly(D,L-lactide-co-glycolide) (PLGA) nanoparticles labeled with Cyanine5.5 amine. The dye was covalently bonded to the carboxylic end group of the polymer exposed on the surface of the previously prepared nanoparticles or, alternatively, the nanoparticles were prepared using the polymer conjugated with the dye.
The modified polymer was synthesized by treating PLGA (Resomer® RG 502H, lactide:glycolide ratio 50:50, viscosity 0.16-0.24 dL/g, molecular weight 7—17 kDa, acid terminated) with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), N,N-diisopropylethylamine, and Cyanine5.5 amine in dichloromethane.

The nanoparticles of both, original and modified polymer were fabricated using a single emulsion method employing a high-pressure homogenizer. In the former case, the obtained nanoparticles were resuspended in MES-buffer (pH 4.7) and treated with EDC and N-hydroxysuccinimide to activate carboxylic groups on their surface, which was then followed by coupling with the dye.
Mean nanoparticle diameters measured by dynamic light scattering ranged from 120 to 150 nm. Relative quantum yields were determined by plotting intensity of fluorescence against absorption for a series of dye solutions and nanoparticles suspensions of various dilution. Relative quantum yields were then calculated using the coefficients of the acquired dependencies. Quantum yields of the nanoparticles were compared to that of a dye solution in ethanol (0.20).
Nanoparticles formulated from the pre-modified polymer demonstrated higher relative quantum yields (0.31) than nanoparticles which were formulated from non-modified polymer and labeled with the dye later (0.04). Thus the first method is preferable for the preparation of the Cy5-labeled PLGA nanoparticles for visualization *in vitro* and *in vivo*.
This work was supported by the Russian Foundation for Assistance to Small Innovative Enterprises within the framework of the international project "Nanoparticles for Brain Use, Diagnostic and Ophthalmological Applications (NABUCO) (application ERA-RUS-14549).

P03

EFFECTS OF CROSSLINKING CONDITIONS ON THE PROPERTIES OF HYALURONIC ACID HYDROGEL

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Hyaluronic acid is a naturally occurring polymer associated with various cellular processes involved in wound healing, Hyaluronic acid also presents unique advantages: it is easy to produce and modify, hydrophilic and nonadhesive, and naturally biodegradable. The aim of this work was to synthesize a biomimetic hydrogel specifically designed to promote tissue repair on the basis of hyaluronic acid (HA). We have studied crosslinking of HA under different conditions in the presence of some reagents and their ratio in to obtain a highly swelling materials.
HA is a water soluble in the entire pH range and forms transparent solutions with high viscosity. To obtain the water-insoluble materials we used 1,4-butanediol diglycidyl ether (BDDE) as a crosslinking reagent. We prepared different BDDE-HA conjugates, which formed a gel when swelling in water. We have studied the physico-chemical characteristics of these materials dependins on the starting HA concentration and the degree of crosslinking, viscosity and extent of hydration.
The porous materials were obtained from the resulting gels by freezing the solvent in a high vacuum. The size, shape and structure of the pores depend on the water content in the initial hydrogel, and the modulus of rigidity is determined by the degree of crosslinking of the HA.
This work was supported by the Ministry of Education and Science of the Russian Federation, project no.7554.

P04

OPTIMIZATION OF WET DIGESTION METHODS FOR THE DETERMINATION OF SILVER NANOPARTICLES ON RICE (*ORYZA SATIVA* L.CV. KDML 105), STICKY RICE (*ORYZA SATIVA* VAR. GLUTINOSA CV. RD 6) AND CHINESE WATER CONVOLVULUS (*IPOMOEA AQUATIC* FORSK. VAR. REPTAN)

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Currently, agriculture is widely which the properties of silver nanoparticles (AgNPs) can be used as anti-bacterial and fungi. AgNPs may be released into the environment and impact on agricultural crops have been exposed. In this work, to investigate the effects of AgNPs to accumulate translocation and impact on three plants including rice (*Oryza sativa* L. cv. KDML 105), sticky rice (*Oryza sativa* var. glutinosa cv. RD 6) and Chinese water convolvulus (*Ipomoea aquatic* Forsk. Var. reptan). In the experiment, the AgNPs were synthesized by pure natural honey as a reducing agent. The characterization and particle size of the silver nanoparticles were evaluated with UV-Vis spectrophotometry and transmission electron microscope (TEM). The results of AgNPs showed the maximum absorption at 423 nm and the particle size in the range of 20-50 nm. In addition, to study on the wet digestion of D1, D2 and D3 methods. The methods of D1 and D2 using nitric acid as a solvent. While the time and temperature are different for digestion on the roots and the shoots of the plants. Then, a D3 method was performed by mixtures of concentrated nitric acid and hydrogen perchloric acid. Moreover, the three plants were exposed with AgNPs to

various concentrations of 0.02, 0.05, 0.1 and 1 mg/L. After wet digestion the AgNPs were determined by Graphite furnace atomic absorption spectroscopy (GFAAS). Therefore, the D2 method was the optimum wet digestion process for the determination of AgNPs in plants, resulting show the percent recovery of between 81.67 to 94.00. While the effect of AgNPs to aggregation and accumulation within the roots and shoots of three plants. The result showed that the roots of sticky rice at a concentration of 0.05 mg/L was accumulated and penetrated to the cell wall and cell in root lead to effect on structural features and transporting nutrients to the plants, including the development on the growth of plant cells.

P05

POLYMERIC NANOPARTICLES BASED ON AMPHIPHILIC PVP FOR DELIVERY OF ACTIVE SUBSTANCES INTO THE CELLS'S NUCLEI

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In this paper, amphiphilic derivatives of poly-N-vinylpyrrolidone (PVP) with different molecular weight, containing one end octadecyl group were used as carriers of biologically active substances (BAS). Polymers were obtained by an original one-step method, varying the content of thiol as a chain transfer agent in the reaction mixture. The structure of polymers was confirmed by ^{1}H and ^{13}C NMR, IR spectroscopy and MALDI TOF mass spectrometry and the values of CMC were determined. The particles of different size were obtained on the basis of amphiphilic polymers by two methods: dialysis and suspension. Nanoparticles with an average diameter about 20 nm were obtained by the suspension method. A corpuscular carrier with an average diameter about 150 nm was obtained by dialysis by free micelle formation without using ultrasonic dispersion. It is shown that nanocarriers based on synthesized amphiphilic polymers are able to include BAS of various nature with high efficiency and deliver them directly to the nucleus of the cell. Curcumin was used as water-insoluble model substance to trace the particles uptake by cell cultures. For in vitro test were used Human primary glioblastoma (U87) and human skin fibroblast (CRL 2429) cell lines. It was shown that the particles based on amphiphilic PVP with a molecular weight of 6 kDa, obtained by the suspension method display curcumin taken up by cells across both glioblastoma and fibroblast cell types, with 5 minutes of exposure to the micelles and fibroblast cells exhibit no difference in curcumin uptake when exposed to endocytosis inhibitors - dynasore, or wortmannin. The drug uptake covers the entirety of the cell in both cell types. Removing the cell nucleus layer shows no difference in intensity, so it is assumed that curcumin enters the cell nucleus. 3D-image of cells shows that the curcumin is distributed across the entire cell in an almost homogeneous fashion. A systematic study showed the prospects of using synthesized amphiphilic polymers of N-vinylpyrrolidone to create nanosized carriers for gene delivery.

P06

POLYMERIC DOUBLE-LAYER NANOPARTICLES BASED ON AMPHIPHILIC PVP FOR CONTROLLED RELEASE OF ACTIVE SUBSTANCES

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Following the emulsion method, we have prepared rifabutin loaded nanoparticles based on amphiphilic PVP derivatives with the second diffusion layer consisting of water-insoluble bioavailable polymer. As a model hydrophobic polymers were used poly(lactic acid) (PLA), polyhydroxybutyrate (PHB) and poly(lactide-co-glycolide)(PLGA). Polymerization of VP proceeds by a free radical mechanism and was carried out under dry argon in the dioxane solution in the presence of initiator, azobisisobutyronitrile and chain-transfer agent (1-Octadecanethiol). Nanoparticles were prepared by using the ultrasonic method, followed by evaporation of the organic solvent (emulsion method). The average size, ζ-potential and particle size distribution of micelles was determined by dynamic light scattering. It was studied that the average size of particles depends on amount of loading drug/water-insoluble polymer, time of ultrasonic dispersion and molecular weight of PVP. The second diffusion layer allows achieve prolonged release of the active substance from the particles compare to the micelles that do not contain it. The particles studied for its rifabutin release behavior in the water at room temperature. It was shown that the sample containing PLA exhibit maximum time release (144 hours) while minimum time release of rifabutin was obtained for particles which does not contain second layer (72 hours). This study concludes that rifabutin loaded particles based on amphiphilic PVP containing water-insoluble polymer as second diffusion layer demonstrate controlled release of rifabutin, extended over a time period of 144 h.

P07

OXIDATIVE DOPAMINE AND N-PHENYLGLICINE POLYMERIZATION

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The kinetics of the oxidation reaction of dopamine and N-phenylglycine by the action of ammonium persulfate in aqueous solution was investigated. Kinetic curves obtained during the investigation of the process of the oxidation of the dopamine under the acidic conditions demonstrate the absence of distinct autocatalytic effect, whereas during the oxidation of N-phenylglycine autocatalytic process takes place. It was shown that the formation of dopamine polymers is possible only under the alkaline conditions, possible mechanism of oxidative polymerization was also proposed. It was found that during the modification of N-vinylpyrrolidoneand ally glycidyl ether copolymer conjugates with ability to solidify in aqueous solutions under the action of oxidants are formed, which can be useful in design and synthesis of bioadhesive systems, suitable among other for gluing soft tissues.

It was found that cation-radicals serve as intermediates during the oxidative polymerization of N-phenylglycine whereas during the oxidative polymerization of dopamine such role is played by appropriate ortho quinines and indoles.

The relationship of polymers of dopamine and N-phenylglycine outcome from synthesis conditions, their solubility in different solvents and their structures were also investigated.

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P08

MULTICELLULAR SPHEROIDS GENERATED FROM STEM CELLS BY RGD-INDUCED CELL SELF-ASSEMBLY FOR TISSUE ENGINEERING

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Mesenchymal stem cells (MSC) are of great interest in tissue engineering due to their ability to differentiate into various cell types for reconstruction of different tissues and organs. Recently, cultivation of MSC in three-dimensional (3D) dense cell aggregates, namely multicellular spheroids, was shown to preserve multilineage potential and to recapitulate hypoxia conditions which are considered as one of key factors of the MSC niche *in vivo*. There are some approaches to generate multicellular spheroids, including handing drop technique, low-adhesive surfaces, microfluidic devices etc. However, mostly all of them suffer from labor-intensity and rather low amounts of spheroids which can be obtained. Therefore, we proposed a simple original RGD-induced cell self-aggregation approach, which has been recently developed by us and reported for tumor cells (Akasov et al. 2016).
The aim of the current study was to develop a novel highly reproducible technique for generation of spheroids from stem cells based on RGD-induced cell self-aggregation, and to evaluate these spheroids as “building blocks” for tissue engineering.
Primary human adipose-derived mesenchymal stem cells and primary normal human dermal fibroblasts were used in this study. The cells were seeded in 96-well plate (10,000 cells per well) and cyclo-RGDfK(TPP) peptide (1-100 μM) was added directly to the monolayer culture. It was shown that both MSC and fibroblasts formed multicellular spheroids with narrow size distribution after adding cyclo-RGDfK(TPP) to monolayer cultures and followed incubation (DMEM+10% FCS, 5% CO_2) for 2-3 days. MSC were able to generate spheroids at minimal lower cyclo-RGDfK(TPP) concentration (10 μM) compared to fibroblasts (25 μM). The viability of cells in the spheroids was confirmed by fluorescent-based live-dead assay. Additionally, to test the spheroid spreading ability in damaged tissue after injection, spheroids were transferred into a 95% collagen I gel derived from rat tails. We have found that spheroids from both primary cultures demonstrated an invasion into the gel after cultivation for 3-5 days. We also suggest that the spheroids could be cultivated as suspension culture or within microcapsules in bioreactor before implantation. Thus, generation of spheroids from stem cells by RGD-induced technique looks promising for tissue engineering applications.
The study was partly supported by the French National Agency (Project ANR-14-CE07-0022).

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P09

HYDROGEL ENZYMES-CONTAINING COMPOSITION FOR WOUND HEALING

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Traditional wound healing materials have wide range of various disadvantages. Most of the solutions that are applied on wound care compositions can be easily dried and inactivated by wound exudates. That is why local application of various solutions of antibiotics, antiseptics and enzymes could be quite ineffective. The other drawback of traditional wound dressings is the only one-way action of such materials: either osmotic (hypertonic solutions, sorbents), antibacterial (antibiotics, antiseptics) or necrolytic (enzymes) etc.
Today the technology of physical and chemical modification of wound healing materials by medicines has been already created. These new forms provide slow drug release in wound and prolonged therapeutic effect. The release of drugs from wound dressing could occur in 48-72 hours, depending on amount of wound exudate. As a result of immobilization of therapeutic agents on polymer material of carriers, it is possible to reduce a therapeutic concentration of drugs, to extend the time of their effectiveness and to limit drug absorption.
The main argument in favor of application of proteolytic enzymes for necrotic wound healing is their possibility to lyse necrotic tissues and to prevent the growth of pathogenic microflora on wound surface.
Selection of an optimal form for a drug and optimal way of its injection into the body are the essential indicators of successful treatment. The absence of interaction between components of healing composition allows to predict the immutability of pharmacological activity of each biologically active compound in medical composition. The creation of systems with targeted drug delivery to the injured tissues is one of the most perspective directions of development of systems with controlled drug release. Such compositions have antibacterial, antioxidant, cleansing, analgesic effect and in combination are a powerful tool in wound treatment.
In this research as model enzymes we studied trypsin (Tr) and chimopsin (Cmp). These enzymes are well studied and widely used in modern medicine in modified and non-modified forms.
We analyzed the effect of polymer matrix: chitosan (Xt), polyacrylamide gel (PAAG), oxypropylmethylcellulose (OPMC), bactericide agent Miramistin (Mir) and anesthetic lidocaine (Ld) on enzymatic activity of Tr and Cmp. The results indicate that in double-systems Ct (in mass ration enzyme:chitosan 1:50), PAAG (1:50) and OPMC (1:100) do not promote a reduction of biological activity of composition. Miramistin (in concentrations up to 20mg) and lidocaine (up to 500 mg) do not affect the studied enzymes in any way.
The thermal inactivation in solution for each component of a wound healing gel was studied.
As a conclusion in this research we showed a possibility of production of enzyme-containing gel based on various polymers with antimicrobial and anesthetic compounds, that are intact to activity of used enzymes during production, storage and exploitation.

P10

N-VINYLPYRROLIDONE AMPHIPHILIC POLYMERS: SELF-ASSEMBLY IN AQUEOUS MEDIA AND INTERACTION WITH BLOOD COMPONENTS

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Current study deals with development, synthesis and properties investigations of novel amphiphilic polymers and copolymers of N-vinylpyrrolidone (PVP), which consist of water-soluble polymeric fragment with controlled molecular weight and one anchor hydrophobic n-alky or di-n-alkyl end group of different design. Such polymers have been shown to form aggregates in water solutions and were able to modify the liposomal membranes.

Here, we present the results of more detailed investigation of self-assembled associates formed by these amphiphilic polymers. In particular, we have studied the influence of the hydrophilic-hydrophobic balance of such polymers on their spontaneous aggregation processes and interaction of the formed self-assembled particles with blood plasma and blood components. With this in mind, polymers have been synthesized, in which the size of the

hydrophilic PVP block varied from 2000 to 10000 Da and the number of methylene groups in the hydrophobic n-alkyl or di-n-alkyl fragment varied within C_6-to-C_{23} interval.

Using the pyrene and 1,6-dyphenyl-1,3,5-hexatriene fluorescent dye probes and dynamic light-scattering method it was shown that the length of both hydrophilic and hydrophobic fragments influences greatly the polymers' ability for self-assembling in aqueous media. Thus, the critical aggregation concentration decreases with increasing the aliphatic radical length and decreasing PVP fragment molecular weight. Using transmission electron microscopy it was shown that obtained aggregates majorly have spherical form, their size (from 30 to 600 nm) and size distribution being depended on the length of hydrophilic and hydrophobic fragments. In physiological solution, the size of aggregates was smaller than in distilled water. Filtration through the porous membranes, ultrasonication and other mechanical impacts did not affect the aggregate size and size distribution noticeably, showing good nanoparticles stability. Also the stability of prepared nanoparticles during long-term storage, freeze-thaw cycles, and in the presence of sodium dodecyl sulfate as a destabilizing agent was confirmed.

It was shown that aggregates of PVP-based amphiphilic polymers were stable in the presence of blood serum and did not affect blood rheological characteristics. Nanoparticles made of amphiphilic polymers with higher molecular weight of PVP hydrophilic part, showed no significant lytic action on ram erythrocytes and no cytotoxicity in regard to human liver hepatocellular carcinoma cells (HepG2) and human embryonic stem cell derived fibroblasts (EBF-H9) both in the trypan blue exclusion assay and the MTT test, thus, demonstrating good amphiphilic PVP nanoparticles biocompatibility.

P11

COVALENTLY CROSSLINKED CHITOSAN HYDROGELS MODIFIED WITH HYALURONIC ACID: PREPARATION AND IN VITRO EVALUATION

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Development of biocompatible biodegradable polymer scaffolds providing cell attachment, growth and proliferation is a priority challenge in regenerative medicine. Natural biodegradable polysaccharides, namely chitosan (Chit) and hyaluronic acid (HA) are promising biomaterials in tissue engineering due to their specific properties. Thus, Chit is often used due to its antibacterial and bio-adhesive activity, while HA being a key extracellular matrix (ECM) component of connective tissue, is involved in cell growth, proliferation and differentiation. Chit and HA can form water insoluble polyelectrolyte complex (PEC). However, since PEC is rather unstable, in order to get stable composite Chit/HA matrices, crosslinking Chit macromolecules could be proposed.
The aim of the study was to prepare composite Chit/HA covalently crosslinked hydrogels and to study their properties, structure and ability to support cell growth in vitro.
Since biological activity of HA is known to depend upon its molecular weight (MW), two HA samples with MW 5 and 30 kDa, respectively, were used in this study. Hydrogels were prepared from HA and Chit (MW 320 kDa) which was crosslinked either with genipin (Gen) or glutaraldehyde (GA). HA was introduced into the hydrogel composition by two ways: before or after Chit crosslinking. When HA was introduced before crosslinking, Chit and HA solutions were mixed at ratio 5:1 (w/w) and incubated with crosslinker solution for 24 h. As a result, the HA macromolecules were distributed within the hydrogel volume. In case of HA addition to the hydrogel after Chit crosslinking, the matrix was just incubated in HA solution (2 wt %) for 2 h. The second approach provided distribution of the HA macromolecules mostly over the hydrogel surface. The macroporous hydrogel structure was obtained by freezing and subsequent lyophilization and was studied by SEM and confocal laser microscopy. Mouse fibroblasts (L929) were cultivated in hydrogels in DMEM medium (10% FBS) for 7 days. Cell spreading, adhesion and growth in the hydrogels were evaluated qualitatively by optical and confocal microscopy, while the number of viable cells was determined by MTT assay. The number of viable cells was found to depend upon the way of HA introduction, and it was higher for all samples where HA was distributed over the hydrogel volume.
The study was supported by Russian Foundation of Basic Research (grant № 15-04-07669 A).

P12

BIODEGRADABLE POLYSACCHARIDE/POLYPEPTIDE MICROCAPSULES FOR DRUG DELIVERY : DESING AND IN VITRO CHARACTERIZATION

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The delivery of anticancer drugs into cells is a priority challenge in biomedicine. A number of drug carriers were proposed for this purpose, such as liposomes, micelles, micro- and nanoemulsions, polymeric microcapsules and nanoparticles etc. A multifunctional drug delivery system based on multilayer microcapsules could be formed by depositing alternating layers of oppositely charged polymers on the surface of non-organic templates. The route of drug administration and the efficiency of the delivery depends on the microcapsule diameter, in particularly submicron size is in demand. The aim of our research was to develop polymeric microcapsules of submicron size for drug delivery and to study their interaction with tumor cells, including their *in vitro* cytotoxicity.
We developed biodegradable polysaccharide/polypeptide microcapsules which can be produced by layer-by-layer technique with following temperature-induced shrinking which allows to decrease microcapsule mean size to 280±90 nm .The microcapsules were composed of 3 layers (dextran sulfate/poly-L-arginine)$_3$ and loaded with doxorubicin. Recently, heat treatment of the polymer film was found to be efficient for some capsule compositions but it has never been employed so far for combination of natural polyelectrolytes. Temperature-induced shrinking was optimized in terms of temperature, composition of a dispersion medium and duration of exposure, in order to reduce microcapsule size and to obtain rather dense shells able to encapsulate low molecular weight compounds. The prepared 280 nm microcapsules were found to be fairly retentive for Rhodamine 6G (MW 479) and doxorubicin (MW 543), while release profiles of these compounds were studied using spectrophotometer at 525 and 490 nm, relatively. Microcapsule incubation at 90°C for 1h caused not only size reduction from 500 to 280 nm but also sterilization of the samples. This approach allowed direct administration without any additional manipulations. Microcapsules uptake, toxicity and antitumor activity of encapsulated doxorubicin were studied in monolayer culture *in vitro* by MTT-test. The internalization efficiency was studied by confocal microscopy and flow cytometry. The developed polyelectrolyte microcapsules are promising drug delivery system.
This work was performed using the equipment of the Shared Research Centre IC RAS and was supported by the Russian Foundation for Basic Research (project 17-33-80141).

P13

MODIFICATION OF POLYHYDROXYALKANOATES FOR IMPROVING THEIR SURFACE PROPERTIES AND BIOCOMPATIBILITY

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Polyhydroxyalkanoates (PHAs) are highly biocompatible, biodegradable and thermoplastic polyesters of microbial synthesis which physical-chemical properties and bioresorption rates can be controlled by obtaining copolymers of different composition. Meanwhile, PHAs are hydrophobic enough, and most available representatives of them are too hard and brittle what restricts their applications.

There are different approaches are developed to increase mechanical and biological PHA properties. They include their mixing with other biopolymers, changing their microstructure by porogen inclusion, treatment of their surface by chemical reagents and chemical modification of the polymer chains.

Mixing poly-3-hydroxybutyrate (P3HB) or poly-3-hydroxybutyrate-*co*-3-hydroxyvalerate with poly-ε-caprolactone has allowed us to obtain a material with increased elasticity in comparison of pure components. Maximal elongation at break values were registered for films containing 5-25% PHAs. Mechanical strength and biocompatibility (which was estimated in cell tests using NIH 3T3 mouse fibroblasts) were preserved or even increased. Additional inclusion of camphor as a porogen followed by its leaching has led to production of polymer films with different porosity.

Treatment of P3HB films with such chemical reagents as oxidizers (H_2O_2), reducers ($NaBH_4$, $LiAlH_4$), hydrolytic agents (HNO_3, $H2SO_4$, NaOH), amines, or step-by-step treatment with NaOH, brominating agents (4-bromobutyryl

chloride, bromine water) and amines has enabled to obtain specimens with increased hydrophilicity and high biocompatibility.

A study of mechanisms of chemical degradation of high-molecular-weight P3HB depending on different conditions (solvents, reagents, temperature) has allowed us to obtain P3HB olygomers with different molecular mass (from thousands to tens of thousands Da) and terminal chemical groups. Mixing aminated olygomers with high-molecular-weight intact P3HB has produced of functionalized "in bulk" polymeric materials possessing increased biocompatibility with appropriate physical-mechanical properties.

The results obtained show that the methods proposed can be applied for increasing surface characteristics and biocompatibility of polymeric scaffolds for tissue engineering.

P14

ENZYME STRUCTURE-ACTIVITY RELATIONSHIP (CHYMOTRYPSIN AND FORMATE DEHYDROGENESE CASES): KINETIC PARAMETERS AND COENZYME SPECIFICITY CAN BE ALTERED UPON APPLICATION OF LOW-FREQUENCY MAGNETIC FIELD

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Application of magnetic nanoparticles for therapy, drug delivery, cells separation and as a contrast agent for MRI has undergone explosive development recently. Nanomechanical approach for biochemical reaction management is one of the promising techniques for using magnetic nanoparticles for biomedical application [1, 2]. In this technique, rotation of magnetic nanoparticles with immobilized on them enzyme under expose of alternative magnetic field is applied for changing an enzyme structure-activity. It was shown previously [1], that in the case of enzyme immobilized between two magnetic nanoparticles (MNP), rotation of MNP under low-frequency magnetic field can significantly change the enzyme structure leading to the decrease of the enzyme-catalyzed reaction rate. Contact forces applied to the enzyme molecule in such nanomechanical "device" amounts to hundreds of pNs [3].

To establish the mechanism of the enzyme activity loss in such nanomechenical "devices" we performed a computer simulation of stretched forces, applied to carboxyl or amino groups on the enzyme surface. As found, the distance between amino-acid residues in the catalytic triad (Ser-His-Asp) was not changed when forces were applied. However, the binding site residues changed their positions dramatically upon application of 160 Hz and 150 mT magnetic field. Kinetic experiment confirmed the results of computer modeling showing 2.5 times increase of Michaelis constant for chymotrypsin immobilized on magnetite@gold nanoparticles under AMF application whereas there were no changes observed in catalytic constant.

Glycine max formate dehydrogenase shows much higher efficiency with NAD as coenzyme than NADP. It was shown in the work presented that coenzyme specificity can be changed at AMF application leading to the significant increase of the rate of NADP reduction (comparing to NAD). Possible reasons being discussed.

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P15

SYNTHESIS OF AMPHIPHILIC POLYMERS OF ACRYLIC ACID AND THE CREATION OF BONE CEMENTS BASED ON THEM WITH ANTI-TUBERCULOSIS ACTIVITY

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Oligomers of acrylic acid were obtained by radical telomerization of acrylic acid in a solution of dioxane under the action of dinitrile-azabisisobutyric acid. Were used mercaptans with different chain lengths as telomerizing agents: hexyl mercaptan, decylmercaptan, hexadecylmercaptan and octadecylmercaptan. The structure of the obtained polymers was studied using 1 H NMR, IR, and UV spectroscopy. The molecular weights of the obtained polymers, ζ-potentials, critical micelle-forming concentrations were determined, and also the isotherms of the adsorption at the water-air interface were recorded.

It was proved that the kinetic order of the initiator in the telomerization of acrylic acid was 0.5, while the order of the monomer concentration is much higher than the theoretical one and was1.6, this was explained by the considerable cooperativity of the chain growth stage due to the association of the monomer in the dioxane solution. It was found that the initiation activation energy has an abnormally low value. This indicates that mercaptan is possibly involved in the redox initiation in the reaction with dinitrilomazobisisobutyric acid.

It was proved that micelles of amphiphilic oligomers of acrylic acid could solubilize the antituberculous drug protionamide. It is possible to obtain cement by subsequent the lyophilic drying and cross-linking of the obtained product with calcium oxide. This cement has considerable strength, contains protionamide. It can be used to seal bone caverns that are formed after surgical removal of the connective tissue affected by tuberculosis.

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P16

EXPERIMENTAL RESEARCH ON STUDYING ETHAMETSULFURON METHYL MIGRATION-PH HARMFUL INDEX IN SOIL-WATER SYSTEM

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Objectives. Study of the pesticide based on ethametsulfuron-methyl exposure on the migration-pH harmful index.

Materials and methods. Ethametsulfuron-methyl (1-methyl-2-[(4-ethoxy-6-metiamine- 1,3,5-triazine-2-yl)carbomaylsulfamoyl]benzoate), sulfonylureas class, as per soil residual life - 1 Class of hazard (highly hazardous compound). The active ingredient and the preparation in terms of the equivalent amount of the active substance in form of water solution were added into upper 20 cm layer of the lysimeters in three-fold replications in the concentrations: the first one complied with the maximal recommended consumption rate (25 g/ha) (1N) - 0.008 mg/kg and 0.0104 mg/kg accordingly; the second one - 10 times below the maximal rate (0.1N) - 0.0008 mg/kg and 0.00104 mg/kg; the third one - 10 times above the maximal rate (10N) - 0.08 mg/kg and 0.104 mg/kg. Determination of ethametsulfuron-methyl concentrations in the water filters were carried out using the method of high performance liquid chromatography with the ultraviolet detector and the diode matrix (0.0005 mg/dm^3).

Results. The experiment proceeded up to reduction of the ethametsulfuron-methyl concentrations in the samples of the lysimetric waters down to its maximum allowable concentration in the water of the water bodies (0.4 mg/dm^3).

After 1-7 days the ethametsulfuron-methyl was not detected in all its concentrations, and in concentration 0.1N the substance was not detected after 8-17 days. In concentration 1N, as of the 8-th day, the active ingredient and the preparation in terms of the equivalent amount of the active substance were detected at the rates 0.0072 mg/dm^3 and 0.0028 mg/dm^3, and accordingly, as of the 9-th day - 0.0073 mg/dm^3 and 0.0035 mg/dm^3, as of the 10-th day - 0.0036 mg/dm^3 and 0.0021 mg/dm^3, as of the 11-17-th days - were not detected in any sample. Concentration 10N (active ingredient): as of the 8-th day - 0.1499 mg/dm^3, the 9-th day - 0.2299 mg/dm^3, the 11-th day - 0.158 mg/dm^3, the 15-th day - 0,0023 mg/dm^3, the 16-17-th days were not detected in any sample. Concentration 10N (preparative form): as of the 8-th day - 0.063 mg/dm^3, the 9 and the 10-th days - 0.049 mg/dm^3, the 12-th day - 0.0024 mg/dm^3,the 13-17-th days - were not detected in any sample.

Conclusions. Based on the obtained results the threshold concentration of the ethametsulfuron-methyl according to the migration-pH harmful index - 0.08 mg/kg (10N), ensuring the substance migration from the soil into the ground waters in the amount safe for human health was determined.

P17

PESTICIDE MUTAGENECITY ASSESSMET

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Some xenobiotics, in particular nanomaterials, can cause specific long-term effects, including mutagenicity. Mutagenic materials can lead to increased incidence of new mutations and genetic burden in human populations, manifested in elevated hereditary pathology; increased incidence of cancer; the violation of reproductive functions; and other undesirable long-term effects. Due to the absence of a universal method that makes it possible to unequivocally assess the ability of a test material to induce different types of mutations in germinal and somatic cells it is necessary to use a battery of methods performed on different test objects. Moreover, the standard research protocols, as well as the set of necessary methods for evaluating mutagenicity, are periodically reviewed in accordance with the acquisition of new knowledge and the development of technologies in this field. In particular, in recent years, some OECD guidelines have been cancelled or revised, and new guidelines have been adopted due to the development of new research methods. Changes in the OECD guidelines for the testing of mutagenic activity of chemicals have affected verification of laboratory proficiency, historical control data, dose levels and dosing schedules, and the sampling required for statistical analysis. In particular, the volume of the analyzed material was significantly increased in the case of cytological methods because of the high probability of false-negative results. At present, we are conducting mutagenicity studies of pesticides, using methods for assessment the induction of gene mutations (Ames test), cytogenetic damage (chromosomal aberrations or micronuclei in mammalian cells in vivo and/or in vitro) and DNA damage (DNA Comet Assay in vitro and/or in vivo), which are harmonized with the new edition of OECD Guidelines. The said battery of tests allows researchers to detect genetic damage at different levels in vitro and in vivo in different organisms. It should be noted, that according to the established practice, testing of mutagenic action of active substances and individual components of the pesticide formulations is conducted. However, in view of recent published data [1-3] and our experimental results on the additive and synergistic mutagenic action of mixtures of active substances of pesticides is favorable to investigate the formulations, that contain two or more active ingredients.

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P18

COMPARATIVE RISK ASSESSMENT FOR METRIBUZIN CONTAINING FORMULATIONS IN NANO- AND MACRODISPERSED FORMS

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Metribuzin-based formulations, widely employed as a systemic herbicide on vegetable plantations, are presented in the nanodispersed form.
Metribuzin, a triazine derivative, is classified as a low-risk compound based on acute oral and dermal toxicity to rats: oral LD_{50} >1000 mg/kg b. w., dermal LD_{50} >2000 mg/kg b. w. It does not irritate skin and conjunctiva in rabbits. No sensitization in guinea pig experiments is observed.
The aim of the present study – to compare risk for workers associated with an application of two forms of metribuzin-based formulations.
Work-conditions were explored for application of formulations by ground tractor spraying of potato plantations with the rate of application for active ingredient: 425 g/ha (the nanodispersed form) and 1470 g/ha (the macrodispersed form).
The risk associated with the application of all metribuzin-based formulations for operators was ascertained to be acceptable (< 1): in terms of exposition, Σ(R) = 0.07-0.03; in terms of dose intake, PR = 0.009 – 0.004.
Metribuzin was detected in the working zone air in the majority of samples with the application of the nanodispersed form, even in the enclosed tractor cabs. Metribuzin was not present on operator's skin.
Resume. For operators, the leading route of absorption for metribuzin-containing formulations in the nanodispersed form is inhalational, for the macrodispersed form – dermal.

P19

SYSTEM FOR NANOMATERIALS ASSESSMENT IN THE RUSSIAN FEDERATION

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Particles of natural origin with the size from single to dozens of nanometers are always present in the environment as components of the air, water, soil, bottom deposits. Significant quantities of these particles also originate from forest fires, volcanic eruptions and from organic fuel combustion. Alongside with these nanoparticles, which appear due to natural processes, nanomaterials (NM) manufactured by artificial means are of increasingly greater significance.

Further development of nanotechnologies and use of NM is limited by possible presence of potentially harmful effects on human health. In this connection, much attention is given to nanosafety problem in the Russian Federation. At present more than 40 regulatory and procedural documents are developed to regulate safety assessment of NM and nanotechnological products.

Wide range of manufactured NM dictates research prioritization. Priority to perform primary toxicological-hygienic and medical-biological experimental studies on NM safety assessment is settled depending on the results of potential hazard identification using method presented in MR 1.2.2522-09 and MR 1.2.0016-10.

Multilevel research strategy was used during development of methods for NM safety assessment. Level 1 – assessment using models of microorganisms' cultures and higher organisms' cells. Level 2 - study using such models, as seeds, sprouts of higher plants, aquatic organisms. Level 3 – toxicological-hygienic study using laboratory animals. Level 4 - study of delayed harmful effects. Documents specifying methods for NM safety testing in biological systems: MI 1.2.2634-10, MR 1.2.2566-9, MI 1.2.2520-09 and etc.

It is necessary to develop new toxicological approaches to evaluate NM safety. The key aspect should be detection of differences in biological effects from NM and similar substances in macrodispersion form. Our studies [1] may be considered as an example of such work showing higher toxicity of the substance in nanoform in comparison with its macrodispersion form.

To solve the problem of NM detection and identification inside human and animal bodies and also in the products and environmental objects several documents were developed, which allow to determine NM content in different environments for the purpose of compliance monitoring and control; evaluate NM allocation in organs and tissues of laboratory animals, detect target organs, the ability of penetration through physiological barriers for the purpose of epidemiological regulation (MR 1.2.2641-10, MR 1.2.2639-10 and etc.).

Thus, at present a unified system of regulatory and procedural documents regulating safety assessment and control of NM at all stages of their life cycle is developed in the Russian Federation.

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P20

THE DETERMINATION 2,4-D IN SOME FOOD PRODUCTS (MILK, EGG, LIVER, KIDNEYS) BY THE METHOD OF TRIPLE QUADRUPOLE UHPLC-MS/MS WITH USED QUECHERS FOR SAMPLE PREPARATION.

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Due to diversity of pesticides used in food protection and globalization of the food industry, the monitoring of programs that cover a large number of pesticides is important. 2,4-D possesses demonstrated cytotoxic and mutagenic effects. Codex Alimentarius Commission of United Nations Food and Agriculture Organization (FAO) and Word Health Organization (WHO) has limited the maximum level of 2,4-D in some food materials. According to this, the following 2,4-D residuals levels are permitted; 0.5 mg/kg in barley, wheat and rye, 2 mg/kg in citrus fruits, 0.05 mg/kg in meat, milk and egg, 0.5 mg/kg in herbs.

The aim this work was the determination 2,4-D in some food products (milk, egg, liver, kidneys) by the method of triple quadrupole UHPLC-MS/MS with used QuEChERS for sample preparation.

All analyses were conducted on UHPLC system combined with the generation triple quadrupole mass spectrometers: "Agilent 1290 Infinity LC, with "Agilent Triple Quad 6460". Column: Agilent ZORBAX Eclipse Plus RRHD C18 (150mm x 2.1 mm x 1.8 µm). The MS-MS analyzer was operated in negative ionization mode and with multiple reaction monitoring to provide maximum specificity and sensitivity in the resolution and detection of fragment masses due to 2,4-D.

A QuEChERS extraction and dispersive SPE method was applied to isolate 2,4-D from the investigation matrix with the addition QuEChERS Extraction Kit (Original) 4 g MgSO4, 1 g NaCl with 50 mL Centrifuge Tube (Agilent Bond Elut, cat No 5982-5550).
Removal of residual water and clean-up of polar residues are performed simultaneously using a dispersive solid-phase (d-SPE) clean-up (mix of sorbents cat.no 5982-5421).
As a results our investigation: LLOQ = 0.005 mg/kg (milk, egg); some by-products of farm animals (liver, kidneys) – 0.05 mg/kg. The maximum residue level (MRL) of pesticide is 10 μg/kg (EC Regulation 396/2005). Extraction performance criteria such as repeatability, recovery (accuracy) and sensitivity were investigated.
The developed methods were applied to milk, egg, some by-products of farm animals (liver, kidneys) from the local market.

P21

DEVELOPMENT OF POLYSACCHARIDES GELS AS ANTITUBERCULAR DRUGS CARRIERS

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The objective of present work is development of natural polysaccharides gels as carriers for controlled release of antitubercular drugs for treatment of tuberculosis. Antitubercular drugs such as isoniazid, ethionamide or rifampicin were used in investigation. Immobilization was carried out by means of salt formation with water-soluble natural polysaccharides such as sodium salts of carboxymethylcellulose, alginic or pectic acid. The interaction of drugs with biodegradable polymers was studied from the viewpoint of thermodynamic behavior. free drug concentration was determined from residual drug concentration; the binding constants were calculated from Klotz equation. The data obtained show that the thermodynamic parameters and their temperature dependence show the existence of electrostatic and hydrophobic interactions between drugs and polysaccharides. The release of drugs into model biological medium at 37°C was studied. The correlation between release rates and binding parameters was determined. It has been shown that dosed release proceeds during 5-7 days and depends on nature of polymers, their concentration, pH of solution and ratio of components. Calcium-alginate gel beads containing antitubercular drugs were synthesized. Release of drug from the alginate gel beads of various mannuronic/guluronic ratios in physiological solution was examined. It was found that discontinuous time of the Fickian diffusion of the drug was followed by a burst release of the remaining drugs. The lag time preceded the burst release depended on mannuronic/guluronic ratio. It was revealed that the burst release of drug was initiated by a swift disintegration of calcium-alginate due to exchange on sodium ions. The antitubercular effect of gels tested using disc diffusion method against a museum strain of *Mycobacterium tuberculosis*. Minimal suppressing concentration for polymeric systems was 0,025 – 0,05 mcg/ml. The tuberculostatic activity of drugs released from the gels show antimicrobial activity identical of low molecular drugs. The efficiency of the tuberculosis treatment by gels was shown in experiments on animals. The results obtained in the present work have shown the possibility and outlook of natural polysaccharides gels as carriers of antitubercular drugs for the delivery systems for prolonging the action of chemotherapeutical agents in tuberculosis treatment.

P22

SYNTHESIS AND NEW ASPECTS OF THE APPLICATION OF BRANCHED POLYVINYL ALCOHOL

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Branched polyvinyl alcohol was obtained by modifying polyvinyl alcohol with epichlorohydrin in an alkaline medium. This polymer is able to dissolve at elevated temperatures in water and has only limited swelling at room temperature. The kinetics of the interaction of polyvinyl alcohol of various molecular weights with epichlorohydrin, as well as the temperature dependences of the rate constants of these reactions were investigated. It is shown that the reaction is controlled by diffusion, and the step of introducing the glycidyl groups into the side chain proceeds much faster than the chain branching process. At the same time, a significant increase of the viscosity of the reaction system over the time is associated with the formation of a physical network, upon reaching a certain molecular mass due to the branching process.

Conditions that make it possible to obtain branched polymers of vinyl alcohol cured by Lewis acids, as well as incapable of solidifying under such conditions have been determined.

The possibility of using materials made from branched polyvinyl alcohol to create hemocompatible films with considerable mechanical strength, as well as anti-spiked systems that are promising for use in bowel surgery, has been established.

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P23

SALT STRESS AND EFFECTIVE MICROORGANISMS

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Every year due to waterlogging and salinization of land falls approximately 500 - 600 thousand hectares of agricultural land. Affected salinity of about 50% of all the irrigated land of the globe.
Salinity stress is composite of stresses. In includes a component of drought or reduced water availability that increases osmotic pressure against which the plant must remove water molecules from the water shell of ions. A second component is sodium toxicity, a physiologically well-documented term that describes the replacement of the essential and less poisonous potassium by sodium ions in enzymes, protein complexes and membranes. The third factor is the ionic imbalances that could be caused by the high levels of Na and Cl, e.g. K uptake or distribution could be impaired by excess Na or high Cl could influence NO_3 or H_2PO_4 uptake or utilization.
It is known, that integrated parameters of a level of a metabolism is growth processes and productivity of plants. In this connection pertinently to remind that at salted a substratum inhibition growth processes (division and a stretching of cells) and rates of development of plants.
When exposed to saline environments, many organisms accumulate intracellularly one or more low molecular weight compounds to levels sufficient to maintain equal water potential with the environment. These compounds are mostly polyols (glycerol, sorbitol, mannitol, pinitol), sugars (trehalose and sucrose), some amino acids (praline and ectoine), some quaternary amine derivatives (glycine betaine, B-alanine betaine)
At present, in many developed countries, with the purpose of the induced increase of stability of plants to various stressful factors of environment, including to salts, used ecological pure fertilizers, for example, effective microorganisms EM-technology (EM –effective microorganisms). One of fertilizer of EM-technology is Russian preparation «Baikal EM 1». It is a unique complex of the various microorganisms – lactic acid, nitrogen fixation, photosynthetic bacteria and yeast. «Baikal EM 1» to improve micro - flora of soil, increases productivity of agricultural crops, raises their quality and safety. For the first time Blinov V.A. found that the drug, "Baikal EM 1" has no mutagenic, teratogenic, carcinogenic, allergenic and pyrogenic effect. EM - technology is the only modern technology that covers all areas of agro-industrial complex: soil, plants, animals, treatment agricultural raw materials, production of ecologically clean products. Microorganisms of a preparation transform elements of a nutrition of plants in assimilability the form, enriches soil and composts with vitamins, amino acids and biologically active substances. The soil becomes friable and well structured.
Our researches with different crops (wheat, barley, beans, tomatoes) in a medium (0.6%) of chloride salinity of the substrate, using a «Baikal EM 1» showed that this microbiological fertilizer to some extent reduces the inhibitory effect of salt on the processes metabolism of plants and improves soil structure.

P24

PREPARATION OF INSULATING FILMS OF NEW CLASS ON THE BASIS OF POLYNAPHTOLIENBENZIMIDAZOLES

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Development of modern radio-electronic technologies provided the necessity of manufacturing composed materials on the basis of light sensitive polymers. Only few from the recommended materials were able to be introduced in manufacture. Thorough perfection of micro-electronic devices requires improvement of photoresist parameters. From this point of view polynaphtoilenbenzimidazoles (PNBI) take special interests as in addition to their unique complex peculiarities (thermo-, heat-, radiation-, quality chemical-resistant), they have such non-traditional as photosensibility.

For obtaining suggested thermoresistant composite films the polymer of amidoimide type is used which differs from used in practice non-organic materials in particular sillicium deoxide with its elasticity and from polyimides with good adhesiveness.

By the authors in the polymer $^{13}C\{'H\}$ NMR on the basis of spectroscopic method the use of developed method of qualitative estimation of developed method of quantitative estimation of naphtalimide and naphtoilenbenzimidazole fragments gives opportunity of photolithography or formation process of certain relief to determine exact time of thermal processing and shade veiling during which swelling and peeling of the polymer do not occur.

It will significantly increase reliability of a device in the operation process. But blocking of anhydride group by the used of aromated deamine makes it more stable and convenient for operation and valid for technology, because its properties correspond the producing requires related to the expire time of composite preservation Besides the reagents used for obtaining the polymer are not explosive and inflammable. Complicated apparatus is not necessary for polymer synthesis and it proceeds in ambient temperature.

Performed research gives opportunity to obtain films and layers with high exploitation qualities in the interval of wide temperature range-500°C on the basis on these polymers.

The coats obtained on the basis on PNBI will far more greatly improve the quality of microelectronic equipment manufacturing nowadays, their reliability, durability and will simplify their manufacture and bring economic effects.

P25

NEW OLIGOMER SYSTEMS THAT CONTAIN HETEROATOMS OF NITROGEN AND OXYGEN

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In this report discusses the synthesis, properties and application of new multicomponent oligomer systems containing nitrogen and oxygen heteroatoms based on maleic anhydride, diethanolamine and **polyoxyethylenesorbitan trioleate**, which are proposed for use as corrosion inhibitors.
The problem of corrosion is closely related to environmental problems, as in the process of corrosive destruction there is a significant pollution of the environment due to spills of technological media in the case of corrosion failures.
The synthesis was carried out by amidation of maleic anhydride with diethanolamine followed by catalytic esterification of the amidation product of **polyoxyethylenesorbitan trioleate**. The first stage of the amidation reaction was carried out at room temperature in aprotic solvents DMF or DMSO. The second stage - the catalytic esterification of the amido acid with **polyoxyethylenesorbitan trioleate** was carried out with heating to 130°C and held at this temperature for 4 hours in the presence of tertiary amines as a catalyst.
The physico-chemical properties of the new oligomer systems are studied and their structure was proved by IR spectroscopy. The IR spectrum contains bands that can be related to amide groups with wave number in the 1640 cm^{-1} and ester groups in 1735 cm^{-1} region.
It has been established that the degree of corrosion protection of the new oligomeric system, that studied on model water-salt solutions and calculated by the loss of mass of steel samples of St3 and 17GS sticks 95% with the optimum reagent consumption.

P26

SYNTHESIS OF PHOSPHATE MONOESTERS BASED ON PRODUCTS OF CHEMICAL RECYCLING OF PET BOTTLES

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In this paper, a new anionic surfactant was obtained by chemical recycling of the PET bottle and further modification of bis(2-hydroxyethyl) terephthalate with polyphosphoric acid. Depolymerization of a PET bottle to a monomer bis(2-hydroxy)ethyl terephthalate was carried out with an excess of ethylene glycol in the presence of a zinc acetate. Synthesis of phosphate monoester was carried out in two stages. In the first stage, the reaction of phosphorylation of bis(2-hydroxy)ethylterephthalate with polyphosphoric acid in an aprotic solvent. In the second stage, neutralization of the polyphosphoric acid into orthophosphoric acid was carried out. Extraction of the phosphate mono ester of bis(2-hydroxy)ethyl terephthalate was isolated with diethyl ether.
The structure and properties of phosphate monoester are proved by modern physicochemical and instrumental methods (FT-IR, ^{1}H, ^{31}P NMR spectroscopy).
The possibility of using obtained phosphate monoester as an anionic surfactant, corrosion inhibitor and scaling inhibitor has been shown. The degree of protection against corrosion and prevention of scaling up to 96%.

P27

THE ASSEMBLY OF POLYMER NANOCAPSULES BY A COMBINATION OF LAYER-BY-LAYER ADSORPTION TECHNIQUE AND THERMO-INDUCED SHRINKING

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During the last decades, polymer-based containers became very promising carriers proposed for drug delivery, especially nanocontainers are in demand. The aim of the research is to develop polymer nanocapsules using combination of layer-by-layer shell assembly and its subsequent thermo-induced shrinking.
The size of the polymer multilayer capsules produced by layer-by-layer technique is predetermined by the size of sacrificial template used for their formation. Vaterite $CaCO_3$ particles are especially favourable for assembly of biodegradable capsules owing to mild conditions of dissolution. The developed 500 nm vaterites were used as templates for assembling multilayer nanocapsules with shells of the following compositions: polystyrenesulfonate/polydiallyldimethylammonium chloride, dextran sulphate/poly-l-arginine, poly-l-lysine/sodium alginate. Previously heat treatment was found to promote shrinking of capsules but it has never been employed so far for a combination of natural polyelectrolytes. Firstly, to characterize the thermal stability and temperature behavior of each polymer thermogravimetry and differential scanning calorimetry (DSC) have been used. All samples of nanocapsules were exposed to heating and the process was optimized in terms of temperature (lying in the region of thermal stability), composition of a dispersion medium and duration of exposure. Since we did not find any characteristic changes on the DSC curves in the region from 50 to 200°C, all the transformations of the capsule shell are likely to be attributed to the increased degree of mobility of polymer chains and charge redistribution between the adjacent layers of the shell, than to a phase transition. To evaluate the stability of nanocapsules a zeta-potential was measured at each stage of capsule preparation. Dextran sulphate/poly-l-arginine combination found to be the most promising owing to good colloidal stability and almost twofold shrinking. Besides the reduction of the capsule size, using an atomic force microscope we observed the thickening of capsule shell, which may increase the encapsulation efficiency of low molecular weight compounds. In short, we developed biodegradable dextran sulphate/poly-l-arginine nanocapsules produced by layer-by-layer technique with following temperature-induced shrinking with the mean size of 280±90 nm.
This work was performed using the equipment of the Shared Research Centre IC RAS and was supported by the Russian Foundation for Basic Research (project 17-33-80141).

P28

3D SCAFFOLDS BASED ON CHITOSAN-G-OLIGOLACTIDES: EFFECT OF STEREOCHEMICAL COMPOSITION

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The study was aimed at preparation of scaffolds for tissue engineering in a form of macroporous hydrogels and microcarriers based on chitosan-g-oligo(L,L-/L,D-lactides) and evaluation of the effect of stereochemical composition of grafted oligolactide fragments on the properties and in vitro biocompatibility of the scaffolds. Graft copolymers of chitosan with oligolactides of various stereochemical compositions were synthesized via solid-state reactive blending and characterized using FTIR-spectroscopy and fractional analysis; the grafting yield was comparable for both copolymers. Three-dimensional scaffolds in a form of macroporous hydrogels and spherical microparticles were prepared using freeze-drying and oil-in-water solvent evaporation techniques. Grafting of oligolactide fragments onto chitosan led to possibility to use the copolymers as a core material for fabrication of spherical microparticles without any emulsifier in aqueous phase. In the case of the copolymers application for the microparticle fabrication the total yield of the microparticles was higher than one in a case of using classical emulsifier in aqueous phase. The yield of the microparticles based on chitosan-g-oligo(L,D-lactide) was higher than one based on copolymers with oligo(L,L-lactide) fragments. SEM microscopy showed formation of highly porous structure of copolymer-based microparticles. Macroporous hydrogels were prepared by freeze-drying of the

copolymer solvents in acetic acid and transformed into insoluble form via thermal treatment. The effectiveness of thermal treatment was evaluated as weight loss of the hydrogels after purification from uncured fragments and was lower for the samples having oligo(L,L-lactide) fragments. The swelling degree of oligo(L,L-lactide)-contained hydrogel was in 2.6 and 3.1 times higher than that for hydrogels based on non-modified chitosan and on chitosan-g-oligo(L,D-lactide). Mouse fibroblasts L929 were used were cultivated on the scaffolds made from the copolymers for 7 days. Cell growth was controlled by optical and confocal microscopy, while the number of viable cells was determined by MTT assay after 7 days of cultivation. All types of the hydrogels were found to support cell adhesion, growth and proliferation. The work was financially supported by RFBR (№ 15-02-06233-a).

P29

CHITIN AND CELLULOSE NANOCRYSTALS AS ANISOMETRIC FILLERS FOR 2D AND 3D MATERIALS

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Nanocrystals derived from polysaccharides, such as chitin and cellulose, possess an anisometric rod-like structure and could be successfully used as nanosized fillers to control morphology and properties of various materials. Due to their natural origin and biodegradability they are particularly interesting for filling of materials for food and medicine applications. Nanocrystals were derived by acidic hydrolysis from crab shell's chitin (marked as NC-Ch) and flax stalk's cellulose (marked as NC-Cel). X-ray analysis showed that both types of nanocrystals had higher degree of crystallinity than initial polysaccharides that confirmed preferred hydrolysis of amorphous regions. Size and morphology of the nanocrystals were analyzed using dynamic laser scattering and atomic-force microscopy. Aspect ratio of the NC-Cel was higher than that of NC-Ch: 1.9 and 1.4, respectively. However, NC-Cel had a higher tendency to aggregate with concentration increase then NC-Ch dispersions. To investigate the effect of nanocrystals nature and content on structure and properties of various types of materials they were used as anisometric fillers for two- and three-dimensional materials made of guar, hydroxypropyl guar, chitosan and chitosan-g-oligolactide copolymers. A study of mechanical properties of films casted from 1 wt. % water solutions guar and hydroxypropyl guar contained 0-10 wt. % of NC-Ch or NC-Cel showed that filling of both type of matrix with 0.5-1 wt. % of nanocrystals led to increase in tensile strength up to 45-88 %, while future increase of nanocrystals content up to 10 wt. % led to significant decrease in the films strength. Scanning electron microscopy of cross-sectioned macroporous 3D samples made from freeze-dried 1 wt. % water solutions of guar or hydroxypropyl guar filled with 1 wt. % of NC-Cel or NC-Ch showed that the samples based on guar possessed more irregular structure than ones made of hydroxypropyl guar. Addition of nanocrystals led to a formation of local defects on wall surfaces in the case of materials made of hydroxypropyl guar, while nanocrystals-contained samples based on guar had anisotropic pore morphology. The effect of nanocrystals loading on properties of chitosan-based materials in a form of films and macroporous hydrogels was evaluated as well. The work was financially supported by RFBR (№ 15-02-06233-a).

P30

THE INFLUENCE OF POTENTIAL DRUG DELIVERY SYSTEMS ON CELLULAR MEMBRANE MODEL AT AIR/WATER INTERFACE

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Organic, inorganic, and hybrid micro- and nanoparticles with complex internal structure are actively studied with the aim of their application for the targeted drug delivery to desired organism regions, organs, or cells. Polyelectrolyte capsules and nanostructured vaterite microparticles are promising systems for this purpose.
In order to use carriers for drug delivery, it is necessary to study their interaction with living cells. Such studies are commonly started from experiments on models of cellular membranes. Langmuir lipid monolayers are widely used as convenient, although simplified, models of biomembranes.
This work focuses on the study of the interaction between different containers and stearic acid and 1,2-dimyristoyl-sn-glycero-3-phosphorylcholine monolayers as biomembrane models.

In case of nanostructured vaterite submicroparticles, according to monolayer compression isotherms, it is clear, that stearic acid seems to bind calcium ions located on the surface of polycrystalline particles. Therefore, the strongest interaction with the monolayer has been observed for unmodified calcium carbonate particles. The particles free of polymer coatings have the strongest effect on the monolayer structure. According to the data, a single polyelectrolyte layer is not always sufficient to control the surface properties of a container consisting of a porous vaterite particle as a core and a polymer coating.
It was shown, that positively and negatively charged oil-contained polyelectrolyte capsules, obtained by ultrasound emulsification [1], prevent the formation of condensed monolayer phase. This effect takes place because of electrostatic interactions between lipid molecules and polyelectrolyte capsules and mutual repulsion of capsules under the monolayer. Interaction of the capsules, modified by polylysine, with condensed monolayer leads to their aggregation under the monolayer. However, this interaction, also, has electrostatic nature and is rather weak due to slight influence on surface pressure and monolayer structure.
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P31

MAGNETITE-GOLD DUMBBELL-LIKE NANOCARRIERS FOR TARGETED DELIVERY AND MRI VISUALIZATION

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During last decades magnetite and gold nanoparticles (NPs) attract a deep interest of scientists due to their potential application in therapy and diagnostics. Fe3O4 NPs have the ability to enhance T2-contrast in magnetic resonance imaging (MRI) and deliver drugs using an external magnetic field. Au NPs are characterized by high stability, biocompatibility, and can be covalently functionalized by a wide spectrum of thiol-containing ligands. The idea of Fe3O4-Au hybrid material creation is the combination of magnetite and gold promising properties as well as the presence of two types of surfaces.
Hybrid magnetite-gold NPs were obtained by the decomposition of iron pentacarbonyl on the surface of gold NPs. As a result, so-called dumbbell-like structures were obtained where magnetite with spherical (sample D-1, 13±2 nm) or cubic (sample D-2, 23±2 nm) shape and spherical gold NPs were connected together pairwise.
The samples were transferred into water by means of block-copolymer Pluronic F127. R2-relaxivity rates at the level of 167 and 385 mM-1s-1 were obtained for samples D-1 and D-2, respectively; the latter value is a record value for hybrid Fe3O4-Au NPs, exceeding the similar characteristics of commercial contrast agents twice. When D-1 and D-2 samples were intravenously administered to Wistar rats at a dose of 7 mg Fe/kg, they effectively increased the contrast of MRI liver images.
The sample D-1 was also used for the selective functionalization of Fe3O4 NPs surface with anti-cancer drug doxorubicin and Au NPs surface – with the ligand of prostate specific membrane antigen (PSMA). Obtained NPs were found to have dose-related toxicity for human prostate cancer cells (LNCaP cell line) and got into the intracellular space after 45 minutes of incubation (according to fluorescence microscopy data). This can be explained by the affinity of the LNCaP cells to the PSMA ligand.
Thereby, in this work magnetite-gold hybrid NPs, which have a strong potential for biomedical application, particularly in targeted drug delivery to liver and prostate cells, and magnetic resonance imaging, were synthesized and characterized. That paves the way to the development of a new theranostic approach.
This work was supported by Grant of Russian Science Foundation 14-13-00731.

P32

BACTERIOLYTIC ENDOLYSINS IMPROVE THEIR EFFICIENCY IN *E.COLI* CELL WALL LYSIS UNDER LOW FREQUENCY MAGNETIC FIELD

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To date, a large number of infectious diseases, the most common among which are diseases caused by gram-negative pathogenic microorganisms, are a global health problem. The traditional method of treatment of infectious diseases caused by the latter is therapy with the use of antibiotics. However, the rapid development of the resistance of pathogenic microorganisms to existing antibiotics forces us to seek an alternative to traditional therapy. One of such alternatives is therapy with the use of preparations containing lysins - bacteriolytic enzymes capable of destroying the bacterial cell wall by hydrolyzing the peptidoglycan, which subsequently leads to the death of the microorganism. Lysins have high specificity for certain pathogens. The main limitation of the use of lysins active towards gram-negative bacteria is the presence of an outer membrane in the latter preventing the penetration of lysin to its substrate. The effectiveness of the penetration of lysin can be increased by destabilizing the outer membrane of gram-negative bacteria.
Theoretical studies of the interaction of functionalized magnetic nanoparticles (MNP) with a biological membrane have shown that the action of ultralow-frequency alternative magnetic field (AMF) can dramatically change membrane permeability.
The purpose of the work presented was to study the effect of ultra-low frequency AMF on the outer membrane of *E. coli* cells interacting with functionalized by dopamine magnetite MNPs. Rod-like (40x10 nm) nanoparticles were used. Electrostatic interactions take place between positive charge of dopamine and negative charge of phosphate groups of lipids. Complex oscillating movements of MNPs occur under AMF application. In this case, oscillating forces and deformations with normal and lateral components appear in the membrane.
It was found out that bacteriophage S394 endolysin and hen egg lysozyme both significantly (2-3 times) increase their catalytic activity in the *E. coli* cell wall lysis in the presence of MNPs under 50 Hz and 68 mT AMF exposure.
Changes in membrane permeability (AMF membrane destabilizing effect) have been confirmed by two independent experiments. In the first one, ß-lactamase release from *E. coli* cell periplasm was revealed upon magnetic field application. As shown, 75% of ß-lactamase could be measured in supernatant of cell suspension after 20 min of AMF application (enzyme concentration in super with no AMF is almost negligible). In the second experiment, the hydrophobic dye Nile Red capable of fluorescing exclusively in nonpolar media (hydrophobic components of cell membrane in our case) was used. As found, the fluorescence intensity of Nile Red sharply decreased ten times upon AMF application. It worth mentioning that fluorescence was coming back after AMF was stopped reaching the initial intensity within 5-7 min (reversible membrane permeability change).
The effect observed could be a promising in development of non-antibiotic antibacterials.
The work was supported by RSF-14-13-00731 grant.

P33

SYNTHESIS AND INVESTIGATION OF THE NANOCAPSULES BASED ON DUMBBELL-LIKE MAGNETITE-GOLD NANOPARTICLES AS DRUG CARRIERS

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Over the past decade magnetic (mostly, magnetite) nanoparticles (MNPs) have been proposed for the use in therapy and diagnostics. However, magnetite NPs are toxic and unstable under physiological conditions. To solve this problem we can prepare hybrid nanomaterials based on Fe_3O_4 and Au, which are stable and biocompatible. Such hybrid nanomaterials can be used as single dumbbell-like NPs or as components of more complex structures, for example, nanocapsules.

The goal of this work is the synthesis and investigation of nanocapsules based on magnetite-gold hybrid nanoparticles. Dumbbell-like nanoparticles were prepared via the decomposition of iron pentacarbonyl over the surface of the gold followed by oxidation under air. Herewith gold nanoparticles were obtained both separately (sample D-1) and during the synthesis (sample D-2). According to the results of TEM all samples had a dumbbell-like structure. In case of sample D-1, magnetite NPs and gold NPs had a spherical shape (Fe_3O_4 13±1 nm, Au 5±1 nm), but in the sample D-2, magnetite had a cubic shape and gold NPs had a spherical shape (Fe_3O_4 23±3 nm, Au 9±2 nm). Both Fe_3O_4 and Au phases were found on X-Ray diffractogram; in addition, NPs had a monocrystalline structure for both samples. By the results of magnetic properties measurement, samples D-1, D-2 had a hysteresis loop, the value of the saturation magnetization was 62, 80 emu/g and coercive force was 13, 60 Oe, correspondingly.

Nanocapsules were made from the self-assembly of polyvinyl alcohol (PVA) with various molecular weight and samples D-1 and D-2. Obtained nanocapsules were investigated by means of DLS and TEM. It is worth noting that the size of nanoparticles and the value of PVA molecular weight influence the ability of nanoparticles to form nanocapsules. In addition, nanocapsules were loaded with DOX and Nile Red. According to the results of fluorescent microscopy, both DOX and Nile Red are present in nanocapsules. Such capsules can be damaged by external alternating magnetic field (U=410 Hz, B=110 mT). Under external magnetic field, maximum DOX release from

nanocapsules was achieved within 3 minutes. Thus, we have synthesized and studied dumbbell-like NPs and nanocapsules that can be used as drug carriers.

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P34

A COMBINATION OF PSEUDOCAPACITANCES OF BOTH THE PANI/MWCNT COMPOSITE ELECTRODE AND THE GEL POLYMER REDOX ELECTROLYTE FOR ENHANCED PERFORMANCE OF THE FLEXIBLE SUPERCAPACITOR

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The problem of energy accumulation and storage is a factor affecting the further development of various technical devices, such as hybrid electric vehicles, mobile electronic devices, solar panels, memory backup systems, medical devices, etc.

Supercapacitors (SCs) currently are the most promising devices for this purpose. A classic SC is an electrochemical capacitor based on the double electric layer in which carbon materials with a high specific surface area are used as electrodes. Such SCs have high power density, however, their energy density is significantly below the energy density of Li-ion batteries. Besides, there is an insufficient cycling stability with such devices in charge/discharge cycles.

In order to improve specific characteristics of the supercapacitor, a combination of pseudocapacitances of both enzymatically synthesized polyaniline/multi-walled carbon nanotubes composite (PANI/MWCNT) and gel polymer redox electrolyte was used. The PANI/MWCNT composite has a core shell structure. The gel of polyvinyl alcohol in sulfuric acid containing sodium 1,2-naphthoquinone-4-sulfonate ($PVA/H_2SO_4/NQS$) was applied as a gel polymer redox electrolyte. The electrochemical studies have shown that the redox behavior of NQS is a diffusion-controlled and quasi-reversible process. The PANI/MWCNT composite in 13 mM NQS solution had a high specific capacitance of ca. 1100 F/g at the scan rate of 5 mV/s. The symmetrical flexible SC device based on the PANI/MWCNT composite and the PVA/NQS gel redox electrolyte had the power density of ca.1.0 kW/kg and the energy density of ca. 28.0 Wh/kg. After 3000 cycles of the potential scanning, the specific capacitance of the SC device decreased by less than 7%.

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P35

CONSTRUCTION OF THE IRREVERSIBLE INHIBITOR OF PLATELET FUNCTIONS BASED ON TAURINE CHLORAMINE

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Introduction: Previously, we discovered the ability of chloramine derivatives of amino acids and taurine (2-aminoethylsulfonic acid) to exert antiplatelet and antithrombotic effects. The use of amino acid chloramines as antiplatelet agents is limited by their low intrinsic stability.

The objective of the work was the development of new stable structural analogues of N-chlorotaurine, which can selectively suppress the functions of platelets in the blood.

Methods: A quantum mechanical nonparametric method (B3LYP / 6-31G) was used to compute the molecular characteristics of chloramine compounds with known stability and new structural analogues of taurine chloramine. These analogues were created by substituting the hydrogen atom of the amino group by certain structures and introducing alkyl groups into the ethane part of taurine. Rate constants for the reactions between reduced glutathione (or methionine) and the chloramines studied were determined with UV-spectrophotometry.

Platelet aggregation was induced with ADP and measured with whole blood aggregometer. Rabbit blood was treated with the 0.25-1.0 mM investigated chloramines.

Results: Stable structures by the criterion of the value of their half-life have been found using the multiple correlation equation, in which the calculated charges of the atoms of active chlorine, nitrogen, carbon bonded to the carbon of the C-N group, the bond length between the nitrogen and carbon atoms served as arguments.

The equation predicted the low stability of cyclic chloramines and, on the contrary, the high stability of the analogues of N-chloro-2,2-dimethyltaurine, in the structure of which there is a substituent of the hydrogen atom of the chloramino group. On the basis of computational predictions, stable compounds were synthesized, which are the new structural analogues of N-chlorotaurine. The stability of the chloramines in aqueous solutions has been determined experimentally. It has been established that the synthesized analogues of taurine chloramine react chemically with sulfur-containing (sulfhydryl and sulfide) groups in proteins and have antiplatelet (antithrombotic) activity.

Conclusion: New structural analogues of taurine chloramine have been created, which, judging by the value of the half-life, is distinguished by raised stability. They possess antiplatelet activity and chemoselectivity with respect to sulfur-containing groups of atoms in protein targets.

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P36

PALLADIUM-POLYPYRROLE NANOCOMPOSITES FOR DETECTION OF CARCINOGENIC ALDEHYDE IMPURITIES IN WATER AND ETHANOL

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Electrochemical detection of formaldehyde and especially acetaldehyde in water and ethanol solutions is the most express in-situ environmental friendly method in comparison of currently used methods (chromatography, spectrometry, fluorimetry). Formaldehyde (methanal) and acetaldehyde (ethanal) are pollutant and carcinogenic substances even in small amount so their detection in liquids and air is still actual analytical and environmental task.
In our previous works [1,2] we proposed the one-step and one-pot method for synthesis of Pd-polypyrrole composites with reproducible morphology and catalytic properties. In such materials Pd nanoparticles are uniformly distributed inside polypyrrole globules.
In actual work Pd-PPy materials were prepared and tested as electrocatalysts for formaldehyde and acetaldehyde selective oxidation in water and ethanol solutions correspondingly.
Obtained results clearly showed, that Pd/PPy composites are perspective materials for electrooxidation of formaldehyde impurities in aqueous solutions and their sensor properties were carried out. Sensitivity coefficient was 67.6 μA cm^{-2} mM^{-1} in Argon and 32.6 μA cm^{-2} mM^{-1} in oxygen atmosphere. Detection limit was 0.89 μM and 0.96 μM correspondingly.
It was shown also that such materials are sensitive to ethanol in ethanol solutions, so these materials can be perspective also as amperometric sensors to acetaldehyde.
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P37

PARTICIPATION OF NITRIC OXIDE MOLECULE IN THE REGULATION OF CARDIAC FUNCTION

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Objectives. It is well known that nitric oxide molecule is a regulator of various physiological processes due to its high reactivity and free penetration through membranes. Nitric oxide easily binds to thiol groups of different proteins (S-nitrosylation), which results in altered performance of ionic channels and intracellular intermediates, it also reacts with various molecules that contain haem and nonhaem iron. The latter aspect was precisely investigated by A.F. Vanin, who established that binding of nitric oxide in complexes with thiols and iron formed dinitrosyl iron complexes. This compound with glutathione as ligand was created in our centre and named as "Oxacom". It, unlike most nitric oxide donors, exerted long-lasting hypotensive action in rats and monkeys due to gradual release of nitric oxide. The similar effect was observed in healthy volunteers and patients with hypertension crisis. The aim of this study was to detect oxacom action on the tone of coronary vessels as well as on myocardial contractility and relaxability ex vivo and in vivo.
Methods. Ex vivo experiments were performed in isolated rat hearts perfused with Krebs solution, saturated with carbogen. A latex balloon of constant volume was inserted in the left ventricle (LV) and pressure and its derivatives were monitored. In vivo experiments were performed in rats, anesthetized with ketamine (100 mg/kg) and LV and aortic pressures were monitored.
Results. Oxacom (0.01-1.0 μm) dose-dependently relaxed coronary vessels of the isolated rat heart without affecting LV developed pressure as well as myocardial contractility and relaxability. In in vivo experiments oxacom (10 mg/kg) promptly reduced blood pressure by approximately 30% with subsequent slow recovery. The time course of myocardial relaxation constant changed similarly, while contractility index altered quite opposite.
During hypoxia-reoxygenation of the isolated rat heart, oxacom significantly reduced intensity of arrhythmias and improved recovery of the contractile function. In rats with chronic heart failure caused by isoproterenol, the effect of oxacom was similar to that observed in control hearts, but relaxation constant was not decreased, but increased with simultaneous reduction of LV diastolic pressure.
Conclusion. Thus, nitric oxide has been actively involved in the regulation of cardiac contractile function, and its effect is determined by specific conditions of myocardial metabolic status.

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SUPEROXIDE DISMUTASE BASED NANOPARTICLES AND THEIR MODIFICATION BY CHITOSAN FOR OPHTHALMIC APPLICATIONS

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Nowadays, an active search for new drugs for treatment of ocular diseases occurs. For example, uveitis is inflammatory disease of the uvea. Search for drugs to treat uveitis effectively is important. The reason is that in the absence of proper therapy, this disease often leads to blindness.
Oxidative stress plays an important role in the pathogenesis of uveitis, and injection of antioxidants may be effective. Antioxidant enzymes, such as superoxide dismutase (SOD) and catalase, have much more efficiency in comparison with small molecular antioxidants because the enzymes react with the substrate repeatedly. However, administering native enzymes to the eye in the form of eye drops is ineffective due to their rapid clearance. Therefore, it is important to create a drug delivery system that will possess long time of circulation and low immunogenicity.
To achieve this goal, SOD-based nanoparticles covered with chitosan were prepared. Chitosan possesses mucoadhesive properties. The use of such dosage forms allows remaining in the administration site for prolonged times, increasing the local and/or systemic bioavailability of the administered drug.
Briefly, SOD-based nanoparticles were prepared by mixing of SOD solution with protamine and PLE-PEG solutions sequentially after that glutaraldehyde was added. Byproducts were removed by centrifugation through centrifugal filters (100 kDa cut-off). Nanoparticles of 50 nm in diameter with negative charge were obtained. As shown in release experiments using a dialysis container (100 kDa), SOD had been released from nanoparticles more slowly than the native SOD; total activity of nanoparticles was preserved for 24 hours. Long circulation time is expected for SOD-based nanoparticles.
Chitosan (5 kDa) solution (1 mg/ml) was added to SOD-based nanoparticles. As shown, zeta-potential changed from negative to positive values indicating that indeed, SOD-based nanoparticles were covered with chitosan.
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