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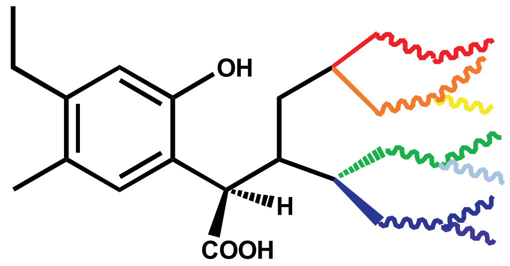
HiT2010 Conference

First International Conference
on Humics-based Innovative Technologies
«Natural and Synthetic Polyfunctional Compounds and Nanomaterials in Medicine and Biomedical Technologies»

Первая международная конференция
по гуминовым инновационным технологиям
«Природные и синтетические полифункциональные соединения и наноматериалы в медицине и биомедицинских технологиях»

Programme & abstracts
Программа и тезисы



November 4-8, 2010, Lomonosov Moscow State University, Moscow, Russia
4-8 ноября 2010 г., МГУ имени М.В. Ломоносова, Москва, Россия

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http://www.humus.ru/hit-2010/

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Non-Commercial Partnership “Center for Biogenic Resources
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Programme

<u>November 4</u> Arrival Day
21:30-24:00 **Excursion "Moscow at night"**

<u>November 5</u>
09:00-10:00 **Registration of the HiT-2010 participants** (lecture hall 446)
Opening of the conference / Welcome addresses (lecture hall 446)
10:00-10:05
Valery V. Lunin, Academician of RAS: Greetings from the Dean of the Department of Chemistry of the Lomonosov MSU
10:05-10:10
Nikolay S. Zefirov, Academician of RAS: Greetings from the Chair of the Organic Chemistry Division and Institute of Physiologically Active Compounds of RAS
10:10-10:15
Oskar I. Koifman, Correspondent Member of RAS, Greetings from the Rector of Ivanovo State Chemical-Technological University
10:15-10:20
Irina V. Perminova, Greetings from the Local Organizing Committee
From Multifunctional Compounds to Multifunctional Drugs
Chairs: Dr. Norbert Hertkorn and Dr. Kamila Kydralieva
10:20-11:00 Invited Lecture
Vladimir V. Bezuglov (Institute of Bioorganic Chemistry of RAS, Moscow)
Hybrid polyfunctional molecules: from concept to multifunctional drugs
11:00-11:40 Invited Lecture
Philippe Schmitt-Kopplin (Institute of Ecological Chemistry, Helmholtz-Zentrum Muenchen)
New insights on molecular processes in environment and health with *metabolomics* tools
11:40-12:10 Coffee break
12:10-12:50 Invited Lecture
Nikolay Melik-Nubarov (Department of Chemistry, Lomonosov MSU)
Synthetic macromolecular modulators of drug activity
12:50-13:30
Irina Perminova (Department of Chemistry, Lomonosov MSU)
On the path forward to biomedical applications of natural polyfunctional compounds - humic substances
13:30-15:00 Lunch
Multifunctional Compounds in Therapy and Prophylaxis of Viral Diseases
Chairs: Dr. Irina V. Sokolova and Dr. Irina Yu. Vashurina
15:00-15:40 Invited Lecture
Edward Karamov (Ivanovsky Institute of Virology of RAMS, Moscow)
New biomedical approaches to fight HIV-AIDs
15:40-16:00
Stephan Kremb (Institute of Virology, Helmholtz-Zentrum Muenchen)
HIV full-replication technology for broad discovery of multiple classes of HIV inhibitors
16:00-16:20
Georgy Krivtsov (Mechnikov Institute for Vaccine Development, Moscow)
Chitosans as prospective adjuvants for vaccine development
16:20-16:30
BashInCom Ltd, Official Sponsor of the HiT-2010
Large-scale production of biologically active humic preparations: Innovative Enterprise "BashInCom Ltd.", Ufa, Russia
16:30-17:00 Coffee Break
17:00-19:00 Poster Session
19:00-22:00 Get-Together Party

November 6

Biological Activity of Humic Substances – Mechanisms and Prospects for Biomedical Applications

Chairs: Dr. Olga Yakimenko and Dr. Istvan Gebefuegi

9:00-9:40 Invited Lecture

Liliya Stepchenko (Department of Veterinary and Animal Physiology, Dniepropetrovsk State Agrarian University, Dniepropetrovsk, Ukraine)

The mechanisms of regulatory influence of biologically active substances of humic nature on the organism of animals

9:40-10:20 Invited Lecture

Alexandrova Olga (Osnabrueck University – Technical University of Ekaterinburg)

Application of a quantum statistical model of humic substances to their influence on reduction of red cell aggregation in blood

10:20-10:40

Natalia V. Yudina (Institute of Petroleum Chemistry, Siberian Branch of RAS, Tomsk)

Biological activity of humic lipids

10:40-12:50 Coffee Break + Internet Break + Poster Session

12:50-13:30 Invited Lecture

Natalia A. Kulikova (Department of Soil Science, Lomonosov MSU, Moscow, Russia)

A New Method for Labeling Humic Substances with Tritium: Future Prospects for Biological Studies

13:30-15:00 Lunch

15:00-15:20

Natural and Modified Humic Substances – Challenging Candidates for Biomedical Applications

Chairs: Dr. Vladimir A. Kholodov and Dr. Elena I. Fedoros

Irina V. Sokolova (Tomsk State University, Tomsk, Russia)

Detoxifying Abilities of Humic Substances: The Influence of UV Pretreatment

15:20-15:40

Yury V. Zhernov (Samara State Medical University, Samara, Russia)

Chemotherapeutic effect of humic substances from low mineralized silt sulphide muds on pathogenic and opportunistic microorganisms

15:40-16:00

Valery N. Babin (Nesmeyanov Institute of Organoelemental Chemistry RAS, “Ultrasun LLC”)

Development of prophylactic-therapeutic system of biologically active food supplement based on restoration of balance in the system “host-microflora”

16:00-16:20

Alexander I. Popov (Saint Petersburg University, Saint Petersburg)

The Removal of Stress at Quails by Humic Substances

16:20-16:40

Tatiana A. Sorkina (Department of Chemistry, Lomonosov MSU, Moscow, Russia)

Synthesis and Application of Iron-Humic Compounds for Correction of Iron-Deficiency State of Plants

16:40-16:45 Sponsor of the HiT-2010

“Medbiopharm” LLC (Obninsk, Kaluga District, Russia)

Center of Innovation Biotechnology and Pharmaceutics Park of Active Molecules (CIBPh "PAM")

16:45-17:15 Coffee Break

17:15-19:30

Panel Discussion

Moderators: Dr. Olga S. Yakimenko and Dr. Istvan L. Gebefuegi

Round Table: “Commercial drugs from polyfunctional compounds: myths and reality”

Moderators: Dr. Alexander I. Popov and Dr. Sergey E. Pigarev

19:30-21:30 Dinner

November 7

In search for molecular structure and biological target of polyfunctional compounds

Chairs: Dr. Schmitt-Kopplin and Dr. Eugene N. Nikolaev

09:00-09:40 Invited Lecture
Norbert Hertkorn (Institute of Ecological Chemistry, Helmholtz Zentrum Muenchen, Germany)
High-precision frequency measurements: indispensable tools at the core of molecular-level analysis for linking non-targeted and targeted metabolomics of complex systems

09:40-10:20
Basem Kanawatti (Institute of Ecological Chemistry, Helmholtz Zentrum Muenchen, Germany)
Fourier Transform Ion Cyclotron Resonance Mass Spectrometry and Computational Tools in Modern Analytical Chemistry

10:20-10:40
Anton Grigoriev (Institute of Biochemical Physics of RAS and Institute of Energy Problem of Chemical Physics of RAS)
Transhumus: Integrated software solution for interpretation of FT-ICR mass spectra of natural organic matter

10:40-11:10 Coffee Break

Poster Presentation Session
Chairs: Natalia V. Yudina and Dr. Nina S. Sedyh

11:10-11:20
Mourad Harir (Institute of Ecological Chemistry, Helmholtz Zentrum Muenchen, Germany)
DCMU complex formation as followed with electrospray Fourier transforms ion cyclotron mass spectrometry

11:20-11:30
Marianna Lucio (Institute of Ecological Chemistry, Helmholtz Zentrum Muenchen, Germany)
Metabolomic study on possible biomarkers of Crohn’s disease

11:30-11:40
Andrey Konstantinov (Department of Chemistry, Lomonosov Moscow State University)
Structural Study of Oxidized Lignin Samples using Size-Exclusion Chromatography, NMR Spectroscopy and Fourier Transform Ion Cyclotron Resonance Mass Spectrometry

11:40-11:50
Gleb Vladimirov (Institute of Biochemical Physics of RAS and Institute of Energy Problem of Chemical Physics of RAS)
Combination of high resolution mass-spectrometry (FT-ICR) and nuclear magnetic resonance (NMR) for analysis of mumijo (shilajit)

11:50-12:00
Michael Kazachkov (Department of Chemistry, Lomonosov Moscow State University)
Optimization of electrospray ionization conditions for more informative mass spectra in structural research of HS for applications in medicine

12:00-12:10
Anastasiya Goldt (Department of Material Science, Lomonosov MSU)
Superparamagnetic Iron Oxide Nanoparticles as MRI contrast agents

12:20-12:30
Alexander Polyakov (Department of Material Science, Lomonosov MSU)
Synthesis of Biocompatible Feroxyhyte (d-FeOOH) Magnetic Nanoparticles Using in situ Stabilization with Polyfunctional Humic Macroligands

12:30-14:00 Lunch

Poster Presentation Session (continuation)
Chairs: Dr. Andrey I. Konstantinov and Dr. Anastasiya E. Goldt
14:00-14:10
Irina Vashurina (Ivanovo State Chemical-Technology University, Ivanovo, Russia)
Prevention of Placental Insufficiency in Rats using Peat Humic Acids
14:10-14:20
Alexander Volikov (Department of Chemistry, Lomonosov MSU)
Synthesis of Adhesive Silanol Derivatives and Their Sorption onto Mineral Support
14:20-14:30
Evgeniy Garanin (Department of Chemistry, Lomonosov MSU)
Synthesis of bioavailable zinc-humic compounds
14:30-14:40
Alexander Popov (Saint Petersburg State University, Saint Petersburg)
Solubilization of Hydrocarbons by Soil Humic Substances
14:40-14:50
Vladimir Kholodov (Dokuchaev Soil Science Institute, RAAS)
Detoxifying Ability of Quinonoid-Enriched Humic Acids with Respect to Copper
14:50-15:00
Olga Philippova (Department of Soil Science, Lomonosov MSU)
Beneficial Effect of Coal Humic Acids and Their Si-enriched Derivative Towards Potatoes and Wheat
15:00-15:10
Nadezhda Sedykh (Department of Veterinary and Animal Physiology, Dniepropetrovsk State Agrarian University, Dniepropetrovsk, Ukraine)
Quantitative Criteria of Biological Activity of New Humics-Based Immunomodulators
15:10-15:20
Polina Zaitseva (Ivanovo State Chemical Technology University, Ivanovo)
Viscous properties of the solutions of copolymers used in the synthesis of polymerporphyrins for medical applications
15:20-15:30
Evgeniy Molchanov (Moscow Aviation Institute (Technical University)
Optimization of production methods of polymeric composite materials based on prepreg technology for precision orthopedic technique
15:30-15:40
Nadezhda Avvakumova (Samara State Medical University)
Modern aspects of medical use of drugs humic peloids sequence
15:40-15:50
Olga Yakimenko (Soil Science Department, Lomonosov MSU)
Comparative Sensitivity of Standard Test–Organisms to Commercial Humates of Different Origin
15:50-16:00 Coffee Break
16:00-17:00
Closing Remarks. Closure of the conference
17:00 Departure from the MSU to the Obraztsov Muppet Theatre and conference dinner

November 8, 2010
10:00-11:00
Reception of the German delegation by the Dean of the Department of Chemistry (TBC)
11:00-12:00
Round Table Discussion: Prospects of Russian-German Cooperation in the field of structure elucidation and biomedical applications of humic substances
12:00-14:00
Excursion in the labs of the Lomonosov Moscow State University
14:00-15:30 Lunch
Departure

Abstracts

Application of a Quantum Statistical Model of Humic Substances to their Influence on Reduction of Red Cell Aggregation in Blood

Olga Aleksandrova[1, 2], Marcus Schulz[3], Michael Matthies[1]
[1]Institute of Environmental Research, Osnabrueck, Germany, oalexand@usf.uni-osnabrueck.de
[2]Federal Ural University, Ekaterinburg, Russia
[3]University of Bremen, Germany

Recently, humic substances (HS) taken from peat and sapropel were shown to be of great value as adaptogens, possessing antineoplastic, anti-oxidant, anti-toxic, and anti-mutagenic characteristics. In vitro, HS decrease red cell aggregation, which is revealed by sampling of HS from definite origin in definite interval of concentration. Therefore, mediating action of HS may arise from promoting a reduction of the aggregation index within definite interval of their concentration, an interpretation which is supported by our quantum statistical model of HS.

Blood plasma represents a colloidal solution containing approximately 90 % water. In blood plasma, red blood cells move separately and are negatively charged. By loss of charge, aggregation is initialized. The addition of HS of definite concentration to blood allows for repairing charge of red cells via binding of negatively charged micelles, which in turn are formed from HS molecules. The transformation of HS molecules to micelles takes place as soon as mass of HS molecule m_{HS} exceeds the critical value $m_{HS\_micelle}$ in the water solution. Due to further increase in HS concentration, a part of micelles are transformed to coagulants. Our quantum statistical model interprets the transformation of HS molecules into micelles and is based on peculiarities of protons, existing as Fermi particles with spin s = 1/2 and possessing a certain magnetic moment μ_p. Binding of micelles to red blood cells is enabled by an excited magnetic state of protons that interact with quinoid or semiquinoid units of proteins of red cell membranes.

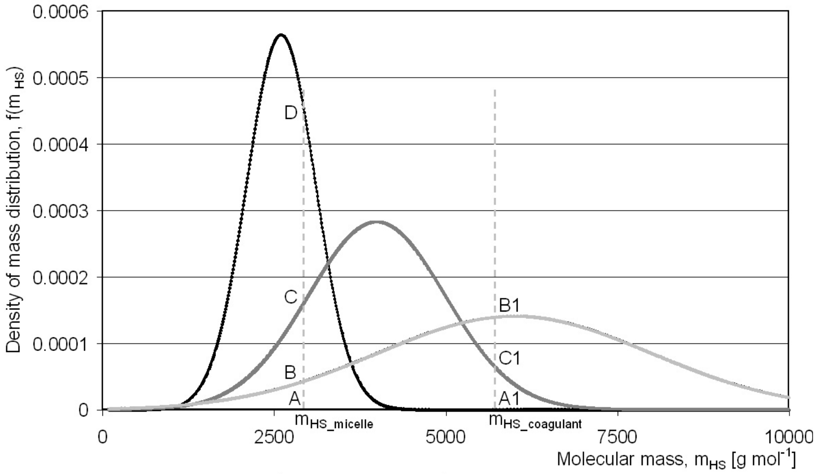

Figure 1. A quantum Gibbs statistical distribution $f(m_{HS})$ of HS particle masses m_{HS} is shown at three different HS concentrations in blood: $C_1 < C_2 < C_3$.

Change of part of micelles in blood plasma was modelled, using a Gibbs canonical distribution method. A phase state of HS molecule as micelle is defined by the mass value m_{HS}, and corresponds to the mass interval ($m_{HS\_micelle}$, $m_{HS\_coagulant}$). In Figure 1, a part of HS micelles is geometrically shown with the area S of surface of curved trapezium under the function $f(m_{HS})$ on the interval ($m_{HS\_micelle}, m_{HS\_coagulant}$). A change in the area *S* of curved trapezium of $S_{ADD_1A_1}$ taken at C_1 to the greater area $S_{ACC_1A_1}$ at $C_2 > C_1$, and subsequently to the smaller area $S_{ABB_1A_1}$ at $C_3 > C_2$, is demonstrated in Figure 1. Thus, when the HS concentration is contained in a definite interval, the concentration of micelles is sufficient in blood to promote their mediating action.

Modern aspects of medical use of drugs humic peloids sequence

Nadezhda Avvakumova
Samara State Medical University. Common, Bioinorganic and Bioorganic Chemistry Department. Samara, Russia navvak@mail.ru

Recent years are characterized by an increasing use of natural humic compounds in various brances industries and agriculture. The use of the humic substances is very important for the modern medicine as curative and preventive purposes. Humanity evoleved in humic background, but the action of the natural macromolecular compounds is not sufficiently studied on the human body. It should be noted that the uniqueness of the humic substances extracted from the medicinal mud. The low salinity mud solution facilitated by the relatively high content of the humic substances. Much moisture makes them expressed biological activity. Recovered hydrogen sulfide breeds gives humic substances pronounced antioxidant properties.

The object of our long-term research is the studying of the physicochemical and biochemical bases of the humic substances in order to get drugs to increase the efficiency of the pelotherapy, save dwindling resources of mud and maintain the ecological balance biogeocenoses zones.

The observation of the structural organization of the humic substances in therapeutic mud by amphiphilic chromatography confirmed their polydispersity. The research result of the humic acids by the capillary electrophoresis mathod makes it possible to place them to the natural polyelectrolytes with different intensity of the charged fragments. The differential thermal mathod identified the occurrence of the polyconjugated aromatic nucleus and the aliphatic peripheral chains with different degree of manifestation of all humic peloids substances. Self-diffusion coefficients have been established by the nuclear magnetic resonance with pulsed magnetic field gradient confirm the closeness, but there is no the identity of the structures these compounds. All preparations behave in aqueous solution as a homogeneous system, they are not isolated components moving at different speeds. The values of self-diffusion coefficients of the investigated material is higher than the pure water, that allows them to predict better permeability through biomembranes.

Studying the biological activity of fulvic, hymatomelanic, humic and humus acids groups in a wide range of concentrations in model of acute carrageenan inflammation discovered a polymodal dependence of all the drugs on the dose. The dynamics of changes of the hematological and immunological parameters by injection of humic acid to animals with the development of adjuvant arthritis reflects a decrease in the inflammatory process with an autoimmune component, and at same time show us the increasing curative action by increasing of course dose.

Based on the totality of the experimental data established that humic peloids substances maintain internal homeostasis of biological systems at the organism, cellular and subcellular levels, contributing to the restoration of physiological functions in pathological conditions and in extreme situations.

Hybrid polyfunctional molecules: from concept to multifunctional drugs

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The predominant paradigm of up-to-date medicinal chemistry is a concept of maximum selectivity of a drug, i.e., its attack being aimed at a single, strictly defined target – Paul Erlich’s “magic bullet”. This paradigm grounds on unbreakable opinion that all chronic pathologies can be divided into elementary diseases-symptoms having distinct targets. As a result almost any patient has a long list of diagnosis and must take daily a huge amount of prescribed drugs. The massive intake of extremely selective pharmaceuticals, that are considered by organism mainly as xenobiotics, disturbs significantly the regulation system of patient’s body. Hence, the likelihood of the appearance of undesired side effects due to the heavy pharmacological load on the metabolic and drug excretion systems becomes higher as well as the risk that the drugs will turn out to be incompatible when used simultaneously. All these facts made scientists to look for an alternative to the “magic bullet“ strategy. As the new paradigm, a concept of `multifunctional drugs' may be considered in which selectivity is opposed to the wideness of pharmacological effect, i.e., the ability of a drug to interact with several targets. Developing this approach we introduced the concept of "Informational drug". This concept based on innovative "Informational-energetic Theory“ founded by Sergey Konovalov (SPb, Russia). According to this Theory a human is mainly an informational entity and his physical body is govered and supported by complex multylayer informational framework. Disturbances in this framework lead to instability of regulatory system, breaks in informational treads and links. Malfunction of informational framework evokes the development of chronic disease that is only one for each patient although it manifests in symptoms’ multiplicity. At physical level all information flows are realized through fluxes of signal molecules. Their excess or shortage is a key component of illness manifestation. The pharmaceutical composed from endogenous signal molecules and capable to correct disturbances in regulation system is termed as “informational drug”. The important feature of informational drug is absence in its structure of molecular fragments foreign to the organism. Just now we are at the very beginning of understanding how to construct real informational drugs. However some productive approaches may be proposed. One of them – design of hybrid polyfunctional molecules. The simplest construction of hybrid molecule is combination of two molecular fragments with distinct but unidirectional biological activities. An example of these type molecules is nitric oxide donating binary drugs like NO-aspirin and other NO-NSAIDs, as well as NO-prostaglandins. Prostaglandins as a natural polyfunctional lipid signal molecules are convenient template for hybrid drugs. Recently discovered in mammalians prostaglandin glycerol esters and amides with ethanolamine (prostamides) or glycine clearly demonstrated that the Nature exploit hybridization approach to produce new signal molecules. Another example came from endocannabinoid field. All endocannabinoids represents functionalized polyunsaturated fatty acids (PUFA). Very promising group is N-acylated dopamines. Using these molecules as a starting template a series of hybrid compounds were synthesized. Combinations of PUFA, GABA and dopamine lead to new hybrid molecules with neuroprotective properties and with ability to improve cerebral circulation. More complex hybrid compounds can be constructed by conjugation of several signal molecules. Very perspective is combination of bioactive lipids and peptides because both types of molecules are essential components of informational framework. We termed these hybrid molecules as “peptolipins” and propose approaches to their synthesis. A separate family of hybrid molecules consists of non-covalent complexes of polyfunctional proteins (like insulin) with natural polymers (e.g. polysialic acid). The work was partially supported by Russian foundation for basic research (project # 09-04-00317).

Synthesis of bioavailable zinc-humic compounds

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Goal of this research was synthesis of humic-based compounds of zinc and to evaluate zinc bioavailability for plants.

Zinc-humic preparations were prepared from commercially available potassium humate and hydrated zinc sulfate. Reaction was carried out with stirring under pH control. Humate dissolution was conducted using ultrasound bath and small amount of alkaline to reach target pH. Then sulfate was mixed with humate solution by dropwise addition. The solid sample was isolated using rotor evaporation. To test reproducibility of the given synthesis, five batches were prepared under the same conditions. The content of zinc in the prepared samples varied from 4 to 5 % (mass).

Biotesting of the obtained preparations was conducted using wheat plants. The plants were grown on Knopp media with and without addition of different zinc sources. As those were used zinc sulfate and five batches of zinc humate synthesized as described above. Bioavailability of zinc was evaluated by the content of zinc in the harvested plants. Determinations of zinc were conducted both in roots and stems. Dry biomass was used for analysis. Prior to determination, the biomass was digested in FOSS Tecator Digester 8. ICPMS technique was used for zinc determination in the obtained solutions. The results are shown in Figure 1.

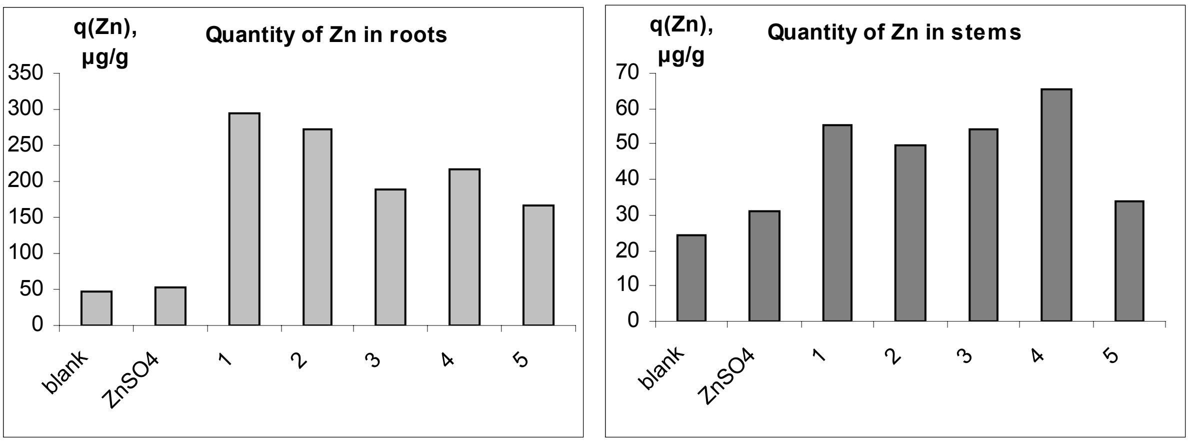

Figure 1. Content of zinc in wheat plants cultivated with different zinc sources: blank - no zinc added; $ZnSO_4$ - zinc sulfate; 1,2,3,4,5 - five different batches of zinc humate.

As it can be seen from Figure 1 the synthesized zinc-humic compounds were bioavailable for plants. In general the roots contained more zinc as compared to stems. Comparison of the zinc content in plants treated with five different batches of zinc humates has shown relatively stable beneficial impact on wheat plants: the content of zinc in treated plants was a factor of five higher as compared to blank for roots, and a factor of 2 - for stems. The obtained results show a good promise for a use of zinc humates as a source of bioavailable zinc suitable both for plant and animal nutrition.

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Microbicides with anti-HIV activity on the base of carbon nanoparticles

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Background:

The most important part of the microbicide development is the search of novel specific anti-HIV-1 agents. The development of nanotechnology has led to significant advances in the field of diagnosis and therapy. In this work we studied water-soluble adducts of carbon nanoclusters (ACNC), active against strains of HIV-1 for the development of safe and effective new microbicides.

Methods:

The cytotoxicity was defined as the viability of T-lymphoblastoid cells CEM SS cultivated at presence of different doses of tested compounds. Anti-viral activity of compounds was defined in modeling HIV-infection using HIV-1_{BRU} strain and CEM SS cells. The virucidal properties of compounds were confirmed by virus inactivation assays.The level of virus reproduction in infected cells at presence of tested compounds was detected with p24 HIV-1 antigen ELISA.

Results:

Adducts of carbon nanoclusters (ACNC) are the new derivatives of carbon, having high solubility in polar solvents, including water. General structural formula of the ACNC: $[OH]_k - [C]_n - (OHSO_2)_m$. All studied adducts of carbon nanoclusters are low-toxic in cells CEM SS. It was shown that these preparations possess inhibitory activity against HIV-infection *in vitro*. 50% effective dose (ED_{50}) of this group of drugs was in the range 21.4-27.5 µg/ml. The study showed that all samples could inactivate the virus, preventing infection of target cells. The most effective was the light fraction ACNC: the adding of 0.75 mg/ml of the drug reduced the level of infection to 0% and the adding of 0.2 mg/ml - decreasing by 98.5%.

Conclusions:

We found that ACNC are very promising for further research (using an extended number of models), for development a candidate microbicides with efficient anti-HIV activity. Taking into account low cytotoxicity of tested compounds, they are promising substances and can be used for HIV-infection prevention with new microbicide preparations, developed on the base of these compounds.

Superparamagnetic Iron Oxide Nanoparticles as MRI contrast agents

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Superparamagnetic iron oxide nanoparticles (SPION) have attracted much attention as a potential multifunctional clinical tool providing cancer detection by contrast – enhanced magnetic resonance imaging (MRI) [1]. Iron oxide nanoparticles γ-Fe_2O_3 and Fe_3O_4 with narrow size distribution are easily synthesized in organic solvents for different applications [2]. However, biomedical applications require that the particles can be readily dispersed in aqueous solutions. To improve their dispersion in aqueous solution, SPION covered different types of water soluble polymers [3].

Here we report a synthesis of hydrophilic SPION capped with amphiflic polymer shells. The prepared nanoparticles were easily coupled with monoclonal antibodies specific to AMVB1 [4]. Thus modified SPION allowed to obtain vessel-specific construction for selective MRI contrast enhancement of tumors that improve the quality of early stage diagnostics and support the control of highly effective therapy.

Superparamagnetic γ-Fe_2O_3 nanoparticles covered by oleic acid with diameter 5-10 nm were obtained [5]. Redispergation of maghemite nanoparticles in chloroform leads to their assembling in nanoclusters of 40-50 nm in diameter. Obtained nanoclusters were covered with amphiphilyc pegylated polymer PMAO-PEG.

The PMAO unit has a hydrophobic octadecene side chain for interacting with the oleic acid surfactant on the iron oxide surface and provides for a free carboxyl functional group for further conjugation to biomolecules including peptides and nucleic acids. The PEG unit enhances the solubility of these SPION in aqueous solutions and circulation time of nanoparticles in blood vessels. The cytotoxic effect of the of PMAO-PEG covered nanoparticles was determined via MTT test on cell cultures of glioma C6, human breast cancer MCF7 and rat's fibroblasts Rat 2. MTT results showed that Fe_2O_3 nanoparticles had no evident cytotoxicity.

Relaxivity values of the obtained nanoparticles was 1370±55 ml/(mg*s) for PMAO-PEG covered particles. These values are up to 10 times higher than for buffer solution. This data confirms that these particles can be used as a MRI contrast agent. ε-NH_2 groups of lysine residues of antibody were used for conjugation of PMAO-PEG covered nanoparticles with antibodies by EDAC or EDC (1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride) as cross-linking agents. Fluorescent microscopy of sections of cerebellum, where rate of expression of AMVB1 is pretty high, showed specific visualization of cerebellum microvessels.

In conclusion, water soluble, biocompatible, stable in biological substances, maghemite nanoparticles were obtained. In vivo MRI experiments confirm our hypothesis about application of nanoparticles as a T2 contrast agent. Nanoparticles conjugation with antibodies to AMVB1 saves binding activity of conjugated antibodies, thus providing a new vector agent for MRI and targeted drug delivery.

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Pharmacological Study of Native Peat Humic Acids

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Search for new raw materials of natural origin BAS for development of new drugs on the basis is an urgent challenge of modern pharmacy. Pharmacological properties and toxicity of native peat HA (pyrophosphate method) have not been studied, also needed the chemical and biological standardization of Civil peats of different genesis. There was a chemical-pharmacognostic research of the chemical structure of native HA representative peat modern methods of physicochemical analysis. Based on the identified characteristics of the chemical structure of HA there were developed methods of standardization, identified indicators of their authenticity and quality, drafted pharmacopoeial monograph Enterprise peat humic acids. There were also studied toxicological and pharmacological properties of HA; evaluated acute toxicity of GC at different ways of application, identified target organs, there was also shown a direct cardiotoxic effect of HA.

In experimental models of pathologies there were tested antihypoxia and hepatoprotective properties of HA. They have expressed hepatoprotective activity in acute CCl4-hepatitis. Their prior intragastric introduction prevents the damaging effect of carbon tetrachloride on functional and metabolic and morphological parameters of rat liver.

HA have expressed antihypoxic effect - increasing the life span of animals and reducing mortality in gistotoksicheskoy and hypobaric hypoxia in prophylactic intragastric test in mice.

Effect of 5-fold intragastric test of HA on the lifespan of mice under hypobaric hypoxia, (X ± Δh)

Species	Number of animals	Life span, min (t-Student criteria)	Mortality from hypoxia $(\chi^2 –)$
Control	25	11,88±1,13	92 %
HA (25 mg/kg)	25	13,72±1,24	84 %
HA (50 mg/kg)	25	16,44±1,19*	76 %
HA (100 mg/kg)	25	17,80±1,31*,**	64 %

Note: * – significant difference (p<0,05) in comparison with the control, ** – significant difference (p<0,05) compared with humic acid 25 mg / kg.

Under normobaric hypercapnic hypoxia GC prevent uncoupling of oxidative phosphorylation in mitochondria, normalizing the activity of succinate-and NAD-dependent processes of energy production in mitochondria of brain and liver of mice.

Transhumus: Integrated software solution for interpretation of FT-ICR mass spectra of natural organic matter

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Natural organic matter (NOM) and humic substances in particular are important research objects as they play important roles in the movement of nutrients in ecosystems and are one of the largest carbon reservoirs. NOM is very complex, consisting of thousands of chemically distinct compounds. Ultrahigh-resolution Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) allows characterizing the compositional and structural diversity of NOM. However, there still are problems with interpretation of mass spectra of NOM. Particularly notable is the lack of specialized software tools for automated analysis of NOM mass spectra. The vast array of existing mass spectrometry software was created for proteomics, genomics, forensics, and pharmaceutical applications and is not suitable for specific needs of NOM analysis. We present software *Transhumus*, specifically designed for automated analysis of FT-ICR mass spectra of NOM.

The software aims to incorporate the most recent developments in interpretation of ultrahigh resolution mass spectra and can serve as a platform for further algorithm development. Main functionality is illustrated by Figure 1: you start with a peak list (m/z values), perform preliminary identification to assess mass measurement accuracy and number of compounds, recalibrate the mass spectrum using unambiguous identifications, and then you can explore the structure of the spectrum using the mass difference statistics. In particular, there is an efficient algorithm for charge state determination which doesn't depend on the presence of ^{13}C-isotopologues of compounds in the spectrum. The neutral mass list can then be used to obtain a final set of compound formulas, using both brute force and "formula extension" approaches.

In addition, the software includes means of result visualization (e.g. van Krevelen plots) and is rather fast, which enables its application in large-scale studies.

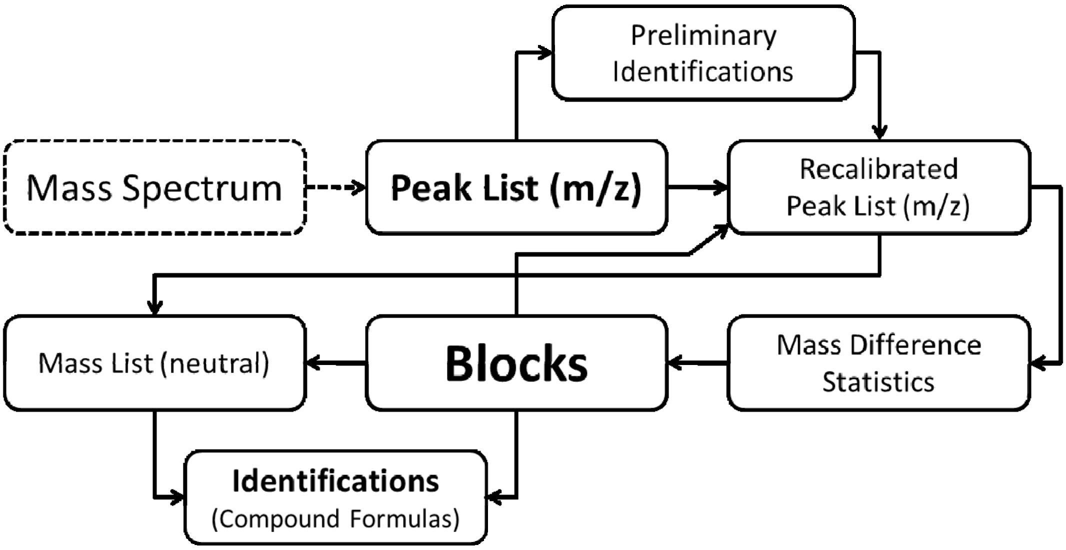

Figure 1. Spectrum interpretation workflow using *Transhumus* software. Boxes show data tables, arrows represent the relation "is needed to obtain" between data. The "Mass Spectrum" box is dashed because peak picking means are not included in *Transhumus*.

DCMU complex formation as followed with electrospray Fourier transforms ion cyclotron mass spectrometry

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Binding of DCMU, a commonly used pesticide from phenyl urea family, on organic amendments as well as aliphatics model compounds was investigated by using high resolution Fourier Transform Ion Cyclotron Resonance mass spectroscopy (FT-ICR-MS) coupled to electrpospray ionization source. Natural (C) and commercial (D) organic amendments were tested at fresh (C_f, D_f) and mature (C_m, D_m) stages parallely with a selected model compunds. Based on the use of electrospray as a ‘soft’ ionization technique in the negative ionization mode, clusters ions containing chlorine atoms were detected and evaluated for DCMU enclosure in both the fresh and mature stages of amendments as well as for the standars. Taking advantage of the high mass resolving power of the ESI-FT-ICR/MS technique, precise sum formulas of the ions which represent a non-covalent interaction between DCMU and aliphatics compounds were unequivocally obtained. Taking into consideration the natural abundance of the chloride isotopes in the DCMU, an algorithm for the detection of DCMU binding, is proposed. Such algorithm enabled the assignment of elemental compositions of DCMU and the chosen amendments as well as the model compounds.

High-precision frequency measurements: indispensable tools at the core of molecular-level analysis of complex materials

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Terrestrial natural organic compounds divide into functional biomolecules which eventually derive from a genetic code, and complex biogeochemical non-repetitive materials, like natural organic matter (NOM) which are formed according to the general constraints of thermodynamics and kinetics from molecules of geochemical or ultimately biogenic origin. While all NOM on earth differs in evolutionary history, the general trajectory of formation and decomposition is constrained by the respective (rather temperate) ecosystem conditions. The combined action of biotic and abiotic reactions characteristic of the respective ecosystems progressively attenuates original biosignatures and the resulting diversity of NOM molecular signatures approaches the limits constrained by the laws of chemical binding. Abiotic, biogeochemical and biological organic molecules occupy vastly different subsets of the chemical space. While the abiotic evolution of natural organic matter often follows entropy-driven trajectories that maximize chemical diversity, carbon based life is confined to a rather restricted biologically relevant chemical space. However, an enormous diversity of large molecules is assembled from a surprisingly small subset of universal precursors and the three-dimensional qualities of these molecules are critical for the sustenance of basic and higher life. Typical biochemical mixtures can be separated into individual compounds which then are assigned by comparison with reference materials (target analysis) or subjected to de novo structural analysis. In contrast, successful molecular-level analyses of NOM often require integration of high-performance separation, high-resolution organic structural spectroscopy and mathematical data treatment.

At present, only high-precision frequency derived data exhibit sufficient resolution to overcome the detrimental effects of intrinsic averaging, which deteriorate spectral resolution of intricate organic materials to an extent of a bulk-type rather than a molecular resolution analysis. Intrinsic averaging is inevitable when the resolution of the method does not adequately match the complexity of the material investigated. It is responsible for the rather restricted bandwidth of low resolution spectral (and bulk) properties of complex organic materials.

High-precision frequency measurements are at the core of the two most influential methods of organic structural spectroscopy for the investigation of complex materials, NMR spectroscopy (provide unsurpassed insight into close-range molecular order) and FTICR mass spectrometry (provide depiction of the compositional space with unsurpassed resolution), and can be translated into isotope-specific molecular resolution detail of unprecedented significance and richness. The currently available discrete analytical volumetric pixel space to describe complex systems (which is defined by NMR, FT mass spectrometry and separation technology) is in the range of 108-14 voxels and therefore capable to provide the necessary detail for a meaningful molecular level analysis of very complex mixtures. This presentation intends to describe the use of complementary high-resolution organic structural spectroscopy to elucidate key structural aspects of various complex organic materials. Studies of natural organic matter like soil-derived, freshwater, marine and atmospheric NOM as well as extraterrestrial NOM have revealed remarkable structural variance which allows detailed conclusions about their formation history.

These in-depth molecular descriptions of NOM in various environments have even emphasized the fundamental differences between abiotic organic synthesis and terrestrial life. In any ecosystem, natural organic matter coevolved with biochemistry and this interaction still defines critical life conditions at the bottom of the food chain. The relationships between abiotic chemical and biological evolution are not yet understood and further research is needed to address these intriguing questions.

Erythrocyte Reversible Aggregation under the Action of Humic Acids

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Blood coagulation was proposed to be used for the assessment of humic acids (Has) coagulation ability. The rheological properties of blood are known to depend substantially on the erythrocyte reversible aggregation. Aggregation parameters may be employed as universal indicators for determining the coagulation and stabilization ability of natural preparations. Among many factors providing blood with coagulability, such coagulants as vitamin K and naphthoquinone derivatives play an important role. Therefore, it is of interest to investigate the influence of HAs, whose macromolecules comprise ordered condensed aromatic nuclei and disordered peripheral moieties containing large amounts of functional groups, on erythrocyte coagulation. The goal of this work is to study erythrocyte reversible coagulation under the effect of HAs isolated from peat subjected to a mechanochemical treatment.

High-moor peat was subjected to a mechanochemical activation in a VCM-10 flowthrough centrifugal vibrating mill (developed at the Institute of Solid State Chemistry and Mechanochemistry, SD RAS, Novosibirsk, Russia) using 10 mm steel balls at an acceleration of 180 m/s^2. Peat was treated both in the absence of chemical reagents and in the presence of NaOH (3%) or celloviridine (CV) (0.5%) as a cellulolytic enzyme. The residence time of the treated materials in the working zone was 2 min.

Erythrocyte reversible aggregation was studied with an instrument using the vibrational photometric method of measuring the optical density in microscopic volumes of blood. Erythrocyte reversible aggregation was investigated in alkaline solutions at HAs concentrations of 0.001 and 0.0001 wt %.

Results of researches have shown that peat mechanical activation increases the yield and solubility of humic preparations. The comparison between results on erythrocyte reversible aggregation testifies that the highest effect is achieved with Has prepared through the peat mechanical activation, either in the absence of additives or in the presence of CV enzyme (fig.1). A rise in the concentration of HAs increases their coagulation ability. The results of the structural analysis enables us to conclude that mechanical treatment enlarges the amount of hydrophilic oxygen-containing fragments and slightly decreases the number of paramagnetic centers in HA molecules.

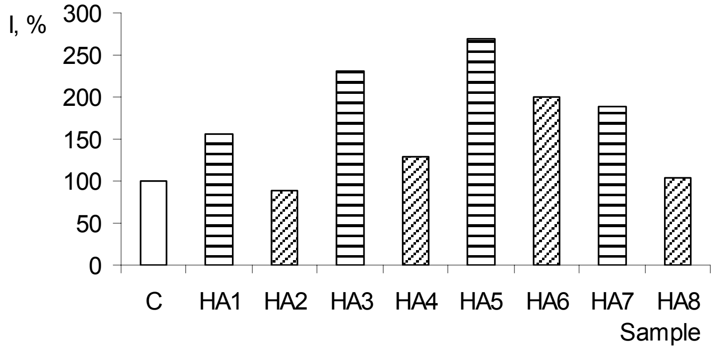

Figure 1. Variations in the index of aggregation as depending on HAs concentration and conditions of peat mechanoactivation: C refers to the control experiment (water);HA1, HA3, HA5, and HA7 refer to HAs (0.001%) extracted from original peat and peat mechanically treated without additives and in the presence of CV (3%) and NaOH (3%), respectively; and HA2, HA4, HA6, and HA8 refer to HAs (0.0001%) extracted from original peat and peat mechanically treated without additives and in the presence of CV (0.5%) and NaOH (3%).

Fourier Transform Ion Cyclotron Resonance Mass Spectrometry and Computational Tools in Modern Analytical Chemistry

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Fourier Transform Ion cyclotron Resonance (FT-ICR) Technique provides the highest mass resolving power for accurate sum formula determinations of ions among all other types of mass analyzers. The combination of non-targeted FT-ICR spectra with advanced statistical tools give out biomarker masses with accurate sum formula determinations. In order to gain details about the structures of the identified biomarkers, experimental MS/MS collision induced dissociation (CID) procedures combined with advanced quantum mechanical calculations are crucial, especially when so standards exist. Here, we shed the light on this issue giving some examples related to organic chemistry. We finally show that this technique does not only allow for sum formula determination and structure elucidation, but it also provides details about possible chemical mechanisms, which explain ion behaviour in the gas phase.

Optimization of electrospray ionization conditions for more informative mass spectra in structural research of HS for applications in medicine

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Humic substances (HS) represent products of postmortal decomposition of living organisms and compose one of the largest reservoirs of organic carbon of global ecosystem. HS possess non-stocihiometric compositions and heterogeneic structures representing natural polyfunctional macromolecular compounds. Comprehension and quantitative description of HS structure belong to one of the most challenging tasks of modern structural analysis. This task is of particular virtue given the prospective pharmacological properties shown both by HS and metal-HS complexes in antiviral, anti-inflammatory, anti-tumor, and other activities. However, a directed use of HS in medicine is greatly hindered due to a lack of single structure which can be prescribed to those compounds for creating quantitative structure-activity relationships (QSAR). New approaches are needed for developing structural descriptors for the compounds of this complexity. Considerable progress in the area of understanding of the molecular structure of HS is currently reached due to application of Fourier transform ion cyclotron mass spectrometry (FTICR MS) in combination with electrospray ionization (ESI). Recently it has been shown by our group that change of electrospray ionization conditions significantly influences on effectiveness of ionization of HS isolated from water [1]. The task of the current work was optimization of electrospray ionization conditions for HS isolated from peat, coal and soil with the goal to obtain the most informative FTICR mass spectrum during relatively short time.

ESI FTICR mass spectra were acquired on the hybrid mass spectrometer LTQ FT Ultra (Thermo Electron Corp., Germany) with 7T superconductive magnet. The instrument was equipped with Ion Max (Thermo Electron Corp., Germany) ion source. Mass spectra processing was performed by original software, developed at the laboratory, which included: solving of Diophantine equations, calculation of total mass difference statistics, determination of charge states.

Dependence of signal intensity on the choice of the solvent, ESI voltage and the sample concentration was studied. The signal intensity was calculated as a summary intensity of 6 peaks from mass spectra, which were reproduced for each solvent and had high enough intensity even at not optimal conditions. As the solvent, methanol, water-methanol mixture (1:3) and acetonitrile were used. For each of chosen solvents the optimal ESI voltage was determined. For the optimal solvent the dependence of the signal from concentration of the sample was studied.

At the result the most informative ESI FTICR mass spectra was acquired for HS dissolved in acetonitrile. For each of measured samples the optimal ESI voltage had been established.

This research was supported by State Contract 16.740.11.0183 of the Ministry of Education and Science of Russian Federation.

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Detoxifying Ability of Quinonoid-Enriched Humic Acids with Respect to Copper

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Catechol, hydroquinone and p-benzoquinone enriched preparations of humic acid (HA) were obtained by modification of HA of brown coal. Modification included phenolformaldehyde condensation of coal HA with catechol, hydroquinone or co-polymerization of HA with p-benzoquinone. All modified HA samples did not expose toxicity to plants.

Detoxifying ability of humic preparations toward copper was estimated using seedling bioassay. Wheat was used as a test plant. Length of 3-day roots was used as a test response.

HA detoxifying ability was quantified using toxicological constants of copper binding to HA are normalized to the organic carbon content in HA preparation (K_{OC}^{tox}) according to the following equation:

$$D = \frac{K_{OC}^{tox} \times C_{HA}}{1 + K_{OC}^{tox} \times C_{HA}}$$

where C_{HA} is a concentration of HA;

D is a detoxification coefficient calculated as follows:

$$D = \left(1 - \frac{R_d - R_{d+t}}{R_d} \middle/ \frac{R_o - R_t}{R_o} \right)$$

where: R_0 – root length of control; R_d - root length in the presence of HA; R_t - root length in the presence of copper; R_{d+t} - root length in the presence of copper and HA.

Modified preparations were shown to possess higher detoxifying ability compared to the initial HA. Detoxifying ability of the quinonoid-enriched humic acids was increasing in the following ascending order: native HA < hydroquinone-enriched HA < catechol-enriched HA < p-benzoquinone-enriched HA.

Thus, the chemical modification of humic substances is a powerful tool for creating new ecologically-sound sorbents aimed to control heavy metal pollution in the environment.

This work has been supported RFBR (grant 10-03-00803-a) and Russian Ministry of Science and Education (state contract 16.740.11.0183).

The Bimodal Effect of Humic Acids in the Blood Coagulation System

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In this study, the term “bimodal effect” is used as a concentration-dependent opposite influence of bioactive substances on the reactivity of a biosystem (1). The objective of the present investigation is to estimate the influence of humic acids (HA) on the early phase of blood coagulation.

Test substances: Selected HS from the Altteich peat deposit (AM) in Eastern Saxony, Germany, were extracted with sodium hydroxide at pH 9, precipitated with 0.1 mol/l hydrochloric acid, oxalic acid and citric acid, respectively. They were washed two or ten times with demine-ralized water. As reference HA, we used the IHSS HA from Waskish Peat and sodium humate (Na humate 76) isolated from the water of a coastal rainmoor near Dierhagen-Neuhaus, Germany.

Method: Thrombelastography (2) was applied for measuring the reaction time (R) in human citrated plasma in presence of HA and in the controls free of HA. The differences of the R-values with and without HA were calculated as n-fold of the control. The R-value is representative of early fibrin formation. It is shortened in **hyper**coagulable and pro- longed in **hypo**coa gulable conditions.

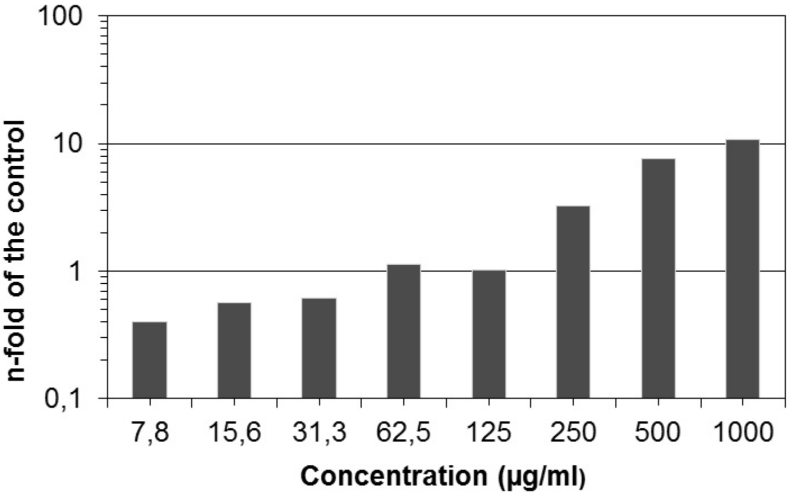

Fig.1: Influence of Na-Humate on the reaction time

Results: AM HA at low concentrations (0.9-31.3 µg/ml) develop a haemostatic effect and act as anticoagulants at higher concentrations (250-1000 µg/ml). These HA effects are independent of the use of different precipitation reagents and the number of washing steps. The reference HA sodium humate 76 react in the same way (Fig. 1). Waskish peat HA at concentrations of 62.5-1000 µg/ml prolong the reaction time, i.e. it develops a strong anticoagulant effect. In contrast, the reaction time was slightly shortened at 15.5 and 31.3 µg/ml HA.

Conclusions: For the first time a bimodal effect of humic acids is demonstrated in blood coagulation. The results of this study are challenging for further investigations to elucidate the mechanism of the observed bimodal effect. The application of HA in low as well as in higher concentrations may open new application fields for selected and profoundly analyzed peat products.

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Natural and Synthetic Humic Substances – Challenging Candidates for Medical Application

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Peat is one of the major resources of natural humic substances (HS), and used for medical purposes since the beginning of 19thcentury. Extraction, isolation and purification of different peat fractions facilitated studies on the chemical composition of HS as well as experimental investigations of peat ingredients in different biosystems. Meanwhile we know that many health-supporting effects of peat such as the specific thermal capacity, the high UV absorption, the binding of causative agents, especially viruses, and the detoxification of heavy metals can be ascribed to its rich content of humic acids (HA).

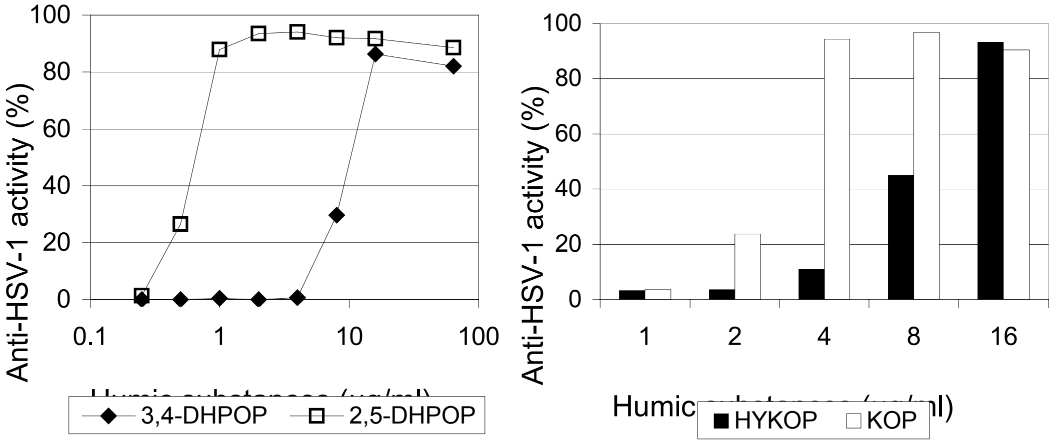

Figure1. Small deviations – strong effect: Anti-HSV-1 activity of HA-like polymers synthesized from diphenolic starting compounds. Left: Influence of OH group positions on antiviral activity. Right: Influence of the double bond in the side chain.3,4-DHPOP, 3,4-dihydroxyphenylacetic acid; 2,5-DHPOP, 2,5-dihydroxyphenylacetic acid; HYKOP, hydrocaffeic acid; KOP, caffeic acid.

The paper presents well-documented interactions of HS with biological systems and addresses the question how synthetic HS assist the researchers in understanding the mechanisms of humic effects. Figure 1 exemplifies the detection of dose-effect relationships between defined phenolic starting compounds and the antiviral effect of synthesized HA on herpes simplex virus type 1 (HSV-1) in Vero cells. The results indicate that the activity of synthetic HA, although they all act as HSV-1 inhibitors, is considerably influenced by structural differences of the starting compounds.

Another important field of preclinical investigations is to examine toxicity of bioactive substances in vitro and in vivo. This aspect deserves more consideration than it have received to date.

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Structural Study of Oxidized Lignin Samples using Size-Exclusion Chromatography, NMR Spectroscopy and Fourier Transform Ion Cyclotron Resonance Mass Spectrometry

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Oxidized lignin is seen as the promising source of humic-like substances which can be used for many biomedical applications. To verify this concept the structural studies on similarity of the structures of oxidized lignin and humic substances (HS) are needed. The objective of this work was to compare the structures of oxidized lignin with those of HS from different sources. For this purpose, both low and high resolution structural analytics was used including size exclusion chromatography (SEC), 1D and 2D NMR spectroscopy and Fourier Transform Ion Cyclotron Resonance Mass Spectroscopy (FTICR MS).

Three samples of oxidized lignin (Nobel ltd, Russia) and two HS (one coal HA from Sakhalin Island, Russia, and one aquatic FA from Suwannee River, USA) were studied.

SEC analysis was conducted using HPLC system consisted of a solvent pump, a packed column, a UV-detector and a PC. The UV-absorbance was measured at 254 nm. The SEC column was 15x250 mm packed with Toyopearl HW-55S (Toso-Haas, Japan). 0,03M phosphate buffer with pH 6,8 was used as a mobile phase at a flow rate of 1 ml/min. The column was calibrated using sodium polystyrenesulfonates.

Quantitative ^{13}C solution state NMR spectra were acquired using Avance 400 spectrometer (Bruker, Germany) operating at 100 MHz carbon-13 frequency. The spectra were recorded on the samples dissolved in 0,3 M $NaOD/D_2O$ at concentration of 80 mg/ml. Two-dimentional COSY, TOCSY and HSQC NMR spectra were acquired using DMX-500 spectrometer (Bruker, Germany) operating at 500 MHz proton frequency. The spectra were recorded on the samples dissolved in 0,1 M $NaOD/D_2O$ at concentration of 12 mg/ml. All NMR spectra were acquired with a 5 mm broadband probe.

FTICR MS analysis was conducted on 12 Tesla spectrometer (Bruker, Germany). The samples were dissolved in methanol and diluted to 10 mg/l. All mass spectra were acquired in negative ion mode.

The NMR data on the content of aromatic and aliphatic carbon have shown the close resemblance between structures of oxidized lignin and those of chernozem soil humic acids. But the size exclusion chromatographic studies have shown that the oxidized lignin samples have much higher hydrophobicity as opposed to all HS samples including those from coal: the recover extent from the column did not exceed 12% while for HS it was not less than 68%. Therefore CH_nO structural fragments of the oxidized lignin samples detected using NMR spectroscopy do not refer to hydrocarbon fragments and belong to ether and ester groups.

The molecular formulas were for the first time determined for oxidized lignin materials using FTICR MS data treated with FIRAN software. It was unambiguously shown that C_3H_2O fragment is a structural block in the analyzed samples as well as $C_6H_4O_2$ and $C_9H_6O_3$. For the first time the stoichiometric coefficients were determined in the formulas assigned to the samples studied. It was shown that the formula can be written as $(C_3H_2O)_x(C_2H_2O)_y(CH2)_z$: where $x \leq 18$, $y \leq 13$, $z \leq 48$.

The conclusion was made that the samples of oxidized lignin analyzed contained two very different classes of compounds: one was of a humic nature containing mostly aromatic compounds, while the other was mostly represented by lipid components like fatty acids.

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Humic substances as active anti-HIV components for microbicides

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Background: At present there is a large data set on ability of humic substances to induce non-specific immune response in living organisms. In particular, anitiviral activity of humic substances have been shown. However, the reported effects are mostly obtained for synthetic humic substances. Systematic studies on antiviral activity of natural humic materials are missing. The objective of this study was to assess anti-HIV activity of a broad set of natural humic materials. The set of humic materials tested included samples of coal and peat humic and fulvic acids as well as non-fractionated materials and more narrow fractions. All samples were isolated and purified in laboratory conditions using standard protocols of International Humic Substances Society (IHSS).

Methods: Anti-viral activity of compounds was defined in modeling HIV-infection using laboratory adapted HIV-1 strains and T-lymphoblastoid cell lines. The level of virus reproduction in infected cells at presence of tested compounds was detected with p24 HIV-1 antigen ELISA. The cytotoxicity was defined as the viability of T-lymphoblastoid cells cultivated at presence of different doses of tested compounds (MTT-test).

Results: All humic materials used in this study showed weak cytotoxicity (10-15%) at concentrations 1,0-1,5 mg/mL. While their anti-HIV activities were high enough. The ED50 values ranged from $3x10^{-3}$ to $4x10^{-2}$ mg/mL. The HIV-activity depended strongly on the source and fraction composition of HS.

Conclusions: Given low cytotoxicity and high efficacy of the humic materials, they can be considered as promising group of compounds suitable for further therapeutic developments. Hence, the performed experiments allowed us to propose new natural humic compounds as active and potent agents for microbicide formulations.

A New Method for Labeling Humic Substances with Tritium: Future Prospects for Biological Studies

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Humics are well known to possess physiological activity in relation to various living organisms including bacteria, algae, fungi, plants, animals, and human beings. Both beneficial and direct toxic effects of humic substances (HS) were reported. However, mechanisms underlying biological properties of HS are still poorly understood. The primary reason for that is a lack of experimental tools for tracking uptake and distribution of natural organic mixtures in living cells and tissues, which makes it extremely difficult to link structure and functions in systems of such high complexity. Besides, predicting HS behavior in biological systems is extremely arduous, as HS are complex mixtures with a number of concurrent properties, such as polyanionic and polyelectrolyte character, hydrophilic and hydrophobic moieties, different functional groups, etc.

The main complication that arises in the study of the interactions of HS with living cells is the lack of a reliable analytical technique for determination of HS in the presence of biomolecules (e.g., proteins, lipids, and saccharides). To overcome the problem, radioactive labeling of HS is being used widely for this purpose. However, the reported studies deal predominantly with synthetic rather than with native humic materials.

A new method for labeling natural HS with the radioisotope tritium (^{3}H) has been developed using humic and fulvic acids of different origin (coal, peat, soil, and water) as the target HS. The method produced HS of high specific activity (0.14-0.63 TBq g^{-1} HS); that permited the tracing of HS at a detection limit of 0.2×10^{-12}g L^{-1} HS. Results from size exclusion chromatography indicated that the tritium label was distributed uniformly over the whole molecular size range of HS as well as no partial decomposition or polymerization of HS occurred during labeling. The performed correlation analysis revealed that there was no any significant relationship between HS properties and specific radioactivity of the obtained ^{3}H-HS. Thus universality of the developed technique for radioactive labeling of HS with tritium could be indicated.

The high specific radioactivity and radiochemical purity allowed direct determination of hydrophobicity and surface activity of humic substances, and investigation of the behavior of humic substances in bacteria, fungi and higher plants including both quantitative estimation of interaction and visualization using tritium autoradiography.

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Prevention of Placental Insufficiency in Rats by Peat Humic Acids

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Placental insufficiency syndrome plays the leading role in the development of perinatal diseases and in ante- and postnatal mortality. Nowadays the expressed tendency is revealed to use bioactive substances of natural origin for correction and prophylaxy of placental insufficiency. It was supposed that natural humic acids (HA) may be effective due to their pronounced antioxidant activity and numerous valuable therapeutic properties.

On the preliminary stage it was showed that HA are not embryotoxic, highten the immune status of rats and fetuses, stimulate physiological development of posterity. The present study was focused on the elucidation of the mechanisms of prophylactic effect of peat HA on placental insufficiency in rats. HA were extracted from lowland peat mined in the centre of European Russia (Kostroma region) as described elsewhere.

Thirty pregnant rats of Wistar line were divided into 3 equal groups: 1st – control, 2nd – the rats with experimental placental insufficiency induced by ligating the uterine arteries, 3rd – the rats with experimental placental insufficiency treated with HA since the first day of pregnancy (the daily dose – 10 mg/kg per os). The initial 1% aqueous solution of HA was poured into a daily amount of drinking water and always fully consumed. After euthanizing rats, one day prior to prospective labors, fetuses, placentas and placental beds were extracted and comprehensive morphological investigation was carried out using light and electronic microscopy and diverse immunohistochemistry procedures.

The obtained results gave undoubted evidence of HA stimulating influence on adaptive processes in uteroplacental region. Placenta tissue and placental bed were well vascularised, the number of blood vessels and their cross-section area were sizably higher than those in the group of untreated placental insufficiency. The increase of the number of utero-placental arteries in placental beds is caused by the expression of vascular endothelial growth factor in trophoblastic and decidual cells. This is apparently attributed to angiogenic effect of HA. The latter favor the completion of gestational reconstruction of arteries of placental bed which is accompanied by the replacement of arteries walls by cytotrophoblastic cells and fibrinoid.

Application of HA led to the reduction of the damage of placental structures. Ultrastructure of trophoblastic cells gave the evidence of a marked protective effect of HA on cytoplasmatic membranes of cellular walls and organellas. Normalizing blood rheological properties HA prevented the disorders of blood circulation, such as thromboses and stases in maternal and fetal vessels. The decrease of expression of prothrombotic Von Willebrand factor in trophoblastic cells is an unambiguous proof of homeostasis restoration.

HA stimulated metabolic processes as could be seen from the increase of the concentration of DNA, RNA and glycogen in trophoblastic, decidual cells and amniotic epithelium. The mean values of body length and mass of fetuses in the 3rd experimental group were statistically the same as in the control group and notably higher than those in the 2nd group. Moreover, application of HA resulted in the decrease of embryonal mortality.

Thus, in the conditions of blood circulation impairment, peat HA prevented placental insufficiency in rats and intrauterine development delay of fetuses. The following basic mechanisms of HA effect can be marked out:

- stimulation of adaptive processes in vascular beds;
- activation of angiogenesis in placentas and placental beds;
- intensification of metabolic processes in placentas;
- cytoprotective effect on trophoblastic cells;
- prevention of uteroplacental blood circulation disorders.

Metabolomic study on possible biomarkers of Crohn's disease

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The metabolomics study applied to disease's evaluation has the main goal to diagnose health and identify factors that cause disease. These studies can lead to enhanced understanding of disease mechanisms Here it is presented a study of the Crohn's disease. The causes and etiology of this disease are currently unknown although both host genetics and environmental factors are thought to play a role. Examination of identical twins with Crohn's disease enabled us to focus on the contribution of metabolites produced by the gut microbiota towards disease status of the host. Ion Cyclotron Resonance Fourier Transform Mass Spectrometry (ICR-FT/MS) was used to discern the masses of thousands of metabolites collected from 17 twin pairs. Pathways with differentiating metabolites included those involved in the metabolism and or synthesis of amino acids, fatty acids, bile acids and arachidonic acid. Several metabolites were positively or negatively correlated to the disease phenotype and to specific microbes previously characterized in the same samples. Our data reveal differentiating metabolites for CD that may provide diagnostic biomarkers and/or monitoring tools as well as insight into potential targets for disease therapy and prevention. The statistical tools start from unsupervised method to lead to supervised methods; such as principal component analysis, partial least square regression, discriminate analysis and a variety of clustering techniques; PCA and hierarchical cluster analysis are now widespread; and they provide an appropriate starting point for further analysis in the interpretation of metabolite profiling. Common to a great part of these methods is that they build up interdependencies between metabolites, relationships between the abundances of the metabolites as revealed by correlation, covariance or distance matrix.

Beneficial Effect of Coal Humic Acids and Their Si-enriched Derivative Towards Potatos

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Pesticides are known to increase agricultural production tremendously as these chemicals act on pests that destroy agricultural produce. However, agricultural pesticide contamination may result from the use and/or misuse of agricultural pesticides, and is manifested as adverse effects on human health. Adverse human health effects or symptoms of agricultural pesticide contamination include headache, body weakness, blurred vision, vomiting, irritability, impaired concentration, abdominal pain etc. Therefore, biological activity of humics is of special interest due to their adaptive properties, that is their ability to mitigate negative environmental effects such as unfavourable weather and soil conditions, presence of toxicants or pathogenic organisms and others.

For this study leonardite HA obtained by desalting of the commercial potassium humate Powhumus (Humintech Ltd., Germany) was used. Si-enriched derivative of HA was synthesized using 3-amino-propyltrimesthoxy-sylane (APTS) according to [1] and assigned as Si-HA. Obtained derivative contained 3.24 % of Si.

To asses biological activity of HA and their Si-enriched derivative field experiments with potatoes *Solanum tuberosum* L. var. Zhukovskii early-ripe was carried out. For tuber treatment 50 mg l^{-1} solutions of HA or Si-HA were used at a dosage 2 l per 100 kg of tubers, and treatment with water was used for the plank. Another two treatments with humics were carried out when mass budding and flowering were observed. Concentration of working solutions for sprinkling was 50 mg l^{-1} and application rate was 40 l per 100 m^{-2}. Trials were performed in quadruplicates.

Performed experiments showed that HA did not influence significantly on plants growth, whereas yield of potatoes treated with Si-HA was 19 c ha^{-1} higher as compared to blank (Table 1).

Table 1. Influence of HA and their Si-enriched derivative on potatoes growth

Variant	Yield, c ha^{-1}	Amount of tubers of different weight, % of total		
		> 80 g	25-50 g	< 25 g
Blank	378±32	33±7	31±6	36±6
HA	383±36	36±8	35±6	29±6
Si-HA	397±32	36±8	33±7	31±7

Alongside with observed increase in total yield of potatoes treated with HA and Si-HA, considerable improvement of external quality of potatoes was observed. In particular, when HA or Si-HA was applied, amount of tubers of larger fraction (tuber weight greater that 80 g) increased, and that of medium fraction (tuber weight 25-50 g) decreased. So, usage of Si-enriched derivatives of humic preparations resulted in both yield and trade quality of potatoes. The letter was evident for topicality of further study of interaction of Si-enriched humic derivatives with crops.

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Mitigating Impact of Coal Humic Acids and Their Si-enriched Derivative on Wheat Yield under Nitrogen Deficiency Conditions

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Nitrogen (N) is often the most deficient of all plant nutrients. However, excessive nitrogen causes lush succulent growth, resulting in greatly increased danger of lodging, delayed maturity and greater susceptibility to diseases. Humic acids (HA) are known to impose beneficial effects on plants, and a wide assortment of humic preparations is now available on the market. Biological activity of HA is of special interest due to bioadaptogenic properties of HA. They are able to mitigate negative environmental effects such as unfavorable weather and soil conditions, presence of toxicants or pathogenic organisms and others. Therefore, development of humic preparations of enhanced adaptive activity is of importance for modern organic agriculture. Silicon seems to be one of the most promising modifying agent which can be incorporated into the structure of HA to enhance adaptive activity. It increases plant resistance under biotic and abiotic stress conditions. In this study we hypothesized that Si-enriched humic preparations will mitigate effect of nitrogen deficiency which is one of the common stresses in plants.

To reach this goal, the silicon-enriched sample of humic materials was synthesized. The commercial potassium humate (Sakhalin Humate, Biomir 2000 Ltd., Russia) (CHS) was used for modification. 3-amino-propyltriethoxy-silane (APTES) was used as a source of Si. The choice of APTES was provided by the presence of reactive amino groups in its structure which can yield amide bonds upon reaction with carboxyl and carbonyl groups. In addition, APTES is commercially available organosilane suitable for preparative production of the corresponding derivatives. Silanol derivatives of CHS were obtained by its condensation with APTES. The reaction was run at ratios 0.2 g of APTES per 1 g of CHS. The corresponding samples was designated as CHS-APTES-20. To perform bioassay, field trials were performed using wheat *Triticum aestivum* L. Experimental design included blank, treatment with CHS and CHS-APTES-20. Wheat was grown according to common practice under adequate supply of nitrogen and without nitrogen application. Humic preparations were applied by means of foliar treatment. The obtained results are shown in Fig. 1.

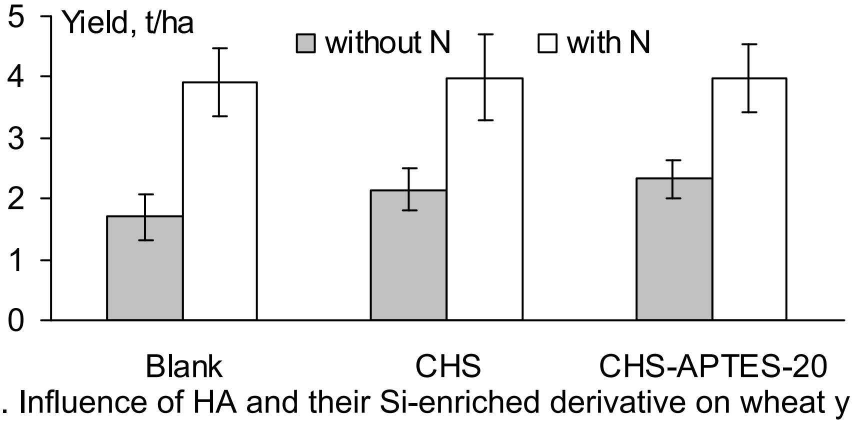

Figure 1. Influence of HA and their Si-enriched derivative on wheat yield under physiological and nitrogen deficiency conditions.

As it can be seen wheat grown with an adequate supply of nitrogen was not influenced by the both humic treatments, whereas a distinctive mitigating effect was observed under N deficiency conditions.

Synthesis of Biocompatible Feroxyhyte (δ-FeOOH) Magnetic Nanoparticles Using *in situ* Stabilization with Polyfunctional Humic Macroligands

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Biocompatible iron oxide and oxyhydroxide nanoparticles represent prospective magnetic materials for biomedical applications such as drug delivery, stem cell tracking, magnetic hyperthermia etc. Uncontrolled aggregation is a serious problem in synthesis and storage of magnetic nanoparticles, which considerably limits their application. This problem can be solved by surface modification of nanoparticles with organic macromolecules, which can prevent or inhibit aggregation. Polyethylene glycol (PEG) and its derivatives are extensively used as stabilizing coatings for magnetic iron oxide nanoparticles. However, PEG molecules coat each nanoparticle separately that leads to high concentrations of polymer needed for particles stabilization. Therefore we suggested using for this purpose humic substances (HS) which represent natural polyfunctional mactoligands with branched structure rich in carboxylic and phenolic groups that allow coating several nanoparticles by each macromolecule.

In the present work *in situ* stabilization of feroxyhyte (δ-FeOOH) magnetic nanoparticles was carried out using humic acids (HA) from coal. δ-FeOOH magnetic phase was chosen due to its layered structure, which allows for incorporation of various ions and molecules into interlaminar spacing resulting in modification of magnetic properties and physiological activity of nanoparticles. The synthesis of nanoparticles included precipitation and rapid oxidation of “green rust” in HA medium. Than nanoparticles were dialyzed and dried in vacuum under room temperature. According to X-ray diffraction data, HA did not slow down oxidation of “green rust” and formation of δ-FeOOH phase. Transmission electron microscopy images (Fig. 1) show that HA effectively prevented aggregation of nanoparticles both during synthesis and after drying. δ-FeOOH nanoparticles stabilized by HA were plate-like with transverse size ~ 20-30 nm and thickness ~ 2-3 nm. The nanoparticles are partially stacked with characteristic interparticle spacing ~ 0.7 nm (Fig. 1a). On the contrary feroxyhyte synthesized without stabilization formed plate-like particles with transverse size ~ 200-300 nm and thickness ~ 30 nm (Fig. 1b).

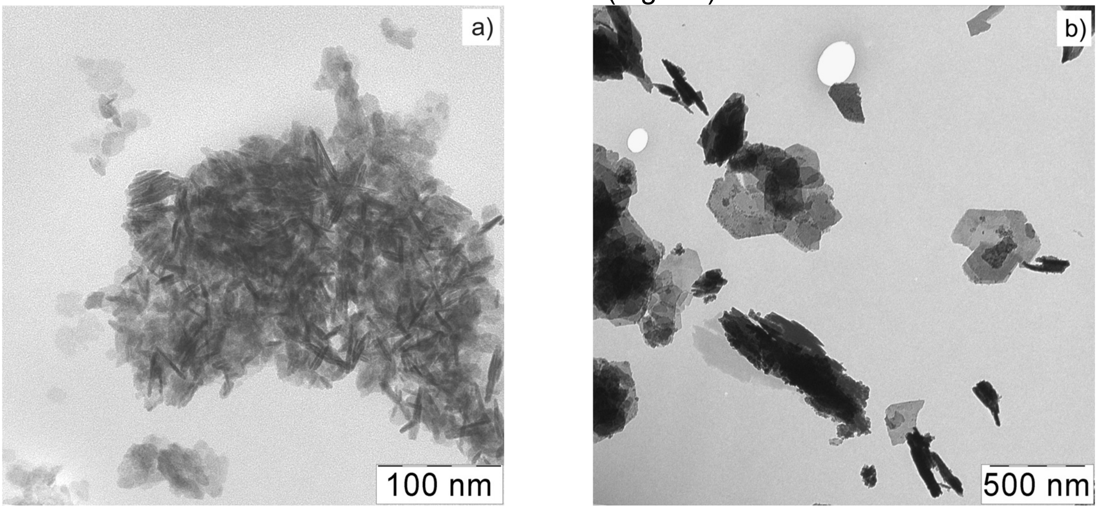

Fig. 1. Transmission electron microscopy images of feroxyhyte (δ-FeOOH) nanoparticles synthesized under different conditions: a) in the presence of HS, b) without HS.

Size reduction of δ-FeOOH crystallites was confirmed by Mössbauer spectra registered at room, liquid-nitrogen and liquid-helium temperatures. These results show a good promise for development of new generation of humic-based magnetic fluids.

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The Removal of Stress at Quails by Humic Substances

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Fowl-like birds or hens (*Galliformes* (Temminck, 1820)) are most sensitive to stress from agricultural animals. When the birds of this order are in a stressful condition, there are decrease of resistance and efficiency, increase of morbidity and murrain that leads to an economic damage.

For the purpose of increase of stress stability, immunity and reparative processes of hens, the ascorbic acid, or vitamin C (*Acidum ascorbinicum*) is very widely used in poultry farming practice. Ascorbic acid regulates blood coagulability, normalizes capillaries permeability, and determines anti-inflammatory and antiallergenic action. However overdose of vitamin C can make negative effect on bird's organism, supply of the oxidised compounds of sulphur to organism particularly. Pharmacological preparations on basis of humic substances (HS) are one of effective stresses-proof-readers, or adaptogens (Zhiljakova, 2006; Safonov, 2007).

The biological activity is one of the important properties of HS, including melanin. Preparations on basis of HS can be used in medicine, veterinary science and animal husbandry as the nonspecific medical product raising resistibility of an organism to influence of various adverse factors. Medical and veterinary preparations on basis of HS are nontoxical (Malama, 1966; Hruleva, 1973; Lotosh, 1991; Parfyonov, Salmina, 1994; the Burayk, Avvakumova, 2003; etc.). Humic substances, extracted from different objects (river waters, peat, composts, soils, brown coal and so forth), raise resistance of chickens-broilers, young growth of a horned cattle and pigs (Tolpa, Chyzhevsky, 1963; Sokrut et al., 1977; Stepchenko et al., 1983; Wu et al, 2000; Filov and Berkovich, 2007; etc.).

The purpose of this paper was revelation of adaptogenic properties of HS (as nano-materials) which were used as biologically active additives.

One-day chickens of quails (*Coturnix* sp.) were selected as research objects. Initially there are two herds: control and test. A livestock of each herd was 300 pieces. The control herd of quails was grown up according to existing technology of farm. The test herd of quails was grown up according to same technology, but biologically active additives on basis of HS and microelements, were added in drinking water. The total term of supervision was 32 days. Four herds have been generated after 15 days: two (control and test) on 60 males (cockerels) and two (control and test) on 100 females (chickens). In all cases chickens contained in cages.

Humic substances have been extracted from earthworm cast compost (vermicompost), which was prepared from cow manure. Humic substances have been modified by macro- and microelements in strictly certain proportion. Solutions of HS had neutral reaction (pH ~ 7) and did not contain helminthes and pathogenic forms of microorganisms.

In result of the spent researches it has been established:

– Biologically active additives on basis of HS raised stress stability of both sexes birds;

– Natural death of young quails has not been registered in case of addition in drinking water of HS;

– The weight of birds (both cockerels, and chickens) control and test groups authentically did not differ;

– Ends of feathers of quails control group (both cockerels, and chickens) have been broken off, whereas feathers of quails test group were whole.

Solubilization of Hydrocarbons by Soil Humic Substances

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Humic substances (HS) have all main properties of colloidal surfactants (Popov, 2004). Surface-active properties of HS are connected with amphiphilic molecule properties. Thus, one site of HS molecules is hydrophilic, and other is hydrophobic (Milanovsky et al., 1993).

One of the main properties colloidal surface-active substances (SAS), including HS, is capacity for solubilization — for intermolecular interaction of a substratum with structured colloidal micelle due to "introduction" of hydrophobic substances into inside micelle.

The solubilization is spontaneous and reversible process. Under solubilization nonpolar hydrocarbons the hydrocarbonic chains in cores of micelles are moved apart, as a result the micelle volume increase. A solubilization of structured colloidal micelle in water solutions usually increases with increase of SAS hydrophobicity and absorbable material hydrophilicity. In result of solubilization the steady disperse systems (corresponding to the sizes of nanomaterial), which are similar to ultramicroheterogeneous emulsions, are formed spontaneously (Frolov, 1988).

As is well known (Frolov, 1988) that inclusion of hydrophobic molecules into colloidal micelles in water solutions depends on nature of these substances. Probably three ways:

1) nonpolar hydrocarbons, introducing into inside micelles, settle down in hydrocarbonic cores of micelles;

2) polar organic compounds (spirits, amines, acids) are built into micelles between SAS molecules so that their polar groups would be directed to water, and lipophilic parts of molecules would be focused to in parallel hydrocarbonic radicals;

3) hydrophobic molecules, for example phenol, do not penetrate to inside micelles, and are fastened onto their surface.

The aim of this paper is estimation of solubilization of HS, which were extracted from different soil types.

As objects of research we had been chose humus-accumulative horizons of six soil types: ordinary chernozem, soddy solod, chestnut typical soil, brown soil, burozem and soddy podsolic soil. Humic substances were extracted from soil humus-accumulative horizons by alkaline sodium pyrophosphate solution (Kononova and Belchikova, 1963). Then structured colloidal HS micelles, stabilized by ions iron, were prepared.

In HS gels it was defined a specific hydrophilic surface by adsorption method with methylene blue (Maslyonkova, Rusakova, 1971). Solubilization effect of HS gels was estimated by quantity of the vaseline oil, which was absorbed by structured colloidal HS micelles.

The carried out researches have allowed:

1) To define a quota HS solubilization — quantity (weight) of vaseline oil, which was absorbed by micelles, on unit of dry HS gels weight;

2) To establish the following number of soils on decrease HS micelles solubilization: soddy solod > brown soil > burozem, soddy podsolic soil > ordinary chernozem > chestnut typical soil;

3) To reveal close inverse essential correlation between the relations of initial humid HS gels weight to vaseline oil volume, which was absorbed by micelles, and a quota HS solubilization;

4) To disclose that value of quota HS solubilization depended on the area of hydrophilic surfaces of structured colloidal HS micelles;

5) To find out that HS gels moisture did not exert influence on solubilization of hydrocarbons.

The obtained results of our research allow to assert that HS can be used as nanocontainers for delivery in a certain place in a live organism of waterproof medical products or to serve as a nontoxical sorbent of hydrophobic substances in an organism of animals or human being.

Collapse of polyelectrolyte star. Theory and modeling

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Collapse of a hydrophobic polyelectrolyte star is considered using Self-Consistend Field theory and numerical Scheutjens-Fleer method. It is shown that by varying such solvent features as Flory-Huggins parameter, pH value and ionic strength, one can get a so called semi-collapsed quasi-micellar star conformation. This structure might be a result of an interplay between hydrophobicity and polyelectrolyte features of the star. As a consequence, microphase segregation appears in the star. Each arm can fall either into a collapsed phase or into a swolen one and thus participate in forming either a dense core or a low-density soluble star corona. A number of arms fallen into collapsed phase depends on the solution features, thus by varying solvent parameters one can control relative sizes of a core and a corona.

This feature can be helpful in drug delivery development. Insoluble in water drug could be delivered within the core into the cell cytoplasm, where the star should either change the conformation or be distructed.

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New insights on molecular processes in environment and health with *metabolomics* tools

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Modern analytical tools enable rapid and sensitive investigations using molecular targeted or non targeted approaches to diagnose status and to monitor therapies within health and environmental sciences. Metabolomic, as the comprehensive study of metabolic reactions is growing very rapidly and integrates the knowledge of earlier developed omics-branches such as genomics, transcriptomics and proteomics.

From a traditional definition in the field of human health, metabolomics measures the concentrations of the large number of naturally occurring small molecules (called metabolites), that are produced as intermediates and end-products of all metabolic processes. They are measured from biological samples and body fluids such as urine, saliva, blood plasma, tissue sample; even the simple breath (exhaled breath condensates) can carry the information about the state of health. In environmental issues the same approach can be followed looking holistically to all small molecules detectable in a given systems in various scales, integrating thus metabolites from living organisms and all their biotic/abiotic transformation products. The total number of different organism metabolites is still unknown; some estimation ranges from a few thousands, up to 200,000 to about 1,000,000, but even this latter estimate may be conservative. Including plant and bacterial metabolites that are not necessary to keep the organism alive, also referred to as secondary metabolites, the number is enormously larger. The probable number of metabolites is also considerably larger than the number of corresponding genes, so it seems that the currently available databases cover at best 2% of the total number of existing metabolites. In environmental organic systems this becomes even more complex as biotic and abiotic diagenetic reaction increase the chemical space and thus chemical diversity. High resolution analytical technology involving separation sciences, spectroscopy and spectrometry for a metabolomic are needed for a description of a system on a molecular level.

Further reading:

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Detoxifying Abilities of Humic Substances: The Influence of UV Pretreatment

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Humic substances (HS) are the products of extremely complex chemical and microbial processes of the decomposition and secondary synthesis of plant and animal residues in soil and water. HS are the main global reservoir of carbon.

Humic substances can decrease or increase bioavailability and toxicity of compounds. Moreover, the photosensitizing and photoquenching effects of humic substances on some chemicals are also known. When HS absorbs UV or solar irradiation, reactive oxygen intermediates are formed which may attack chemicals in the environments and initiate their degradation. Also, the possibility of an UV screening by humic substances on chemicals cannot be excluded since the energy-transfer and charge-transfer between the chemical and humic substances can deactivate the excitated chemical molecules. Therefore, HS can either enhance or decrease the toxicity of compounds after irradiation. Our knowledge about these processes is still very limited.

The aim of this work was to use a bioluminescence test system to study the effect of two factors on solutions of new substituted furocoumarins (sensitizers for phototherapy), phenols and common phenoxyl herbicides: UV radiation from different sources, and humic substances. The toxicity of solutions was assessed using the bioluminescence assay, which is based on lyophilized luminous bacteria *Photobacterium phosphoreum,* produced at the Institute of Biophysics (Krasnoyarsk, Russia). The UV-radiation sources used for investigations were: a DRT-240 high-pressure mercury lamp (Hg) and three barrier discharge excilamps [purchased from the Institute of High Current Electronics of the Siberian Branch, Russian Academy of Sciences]. These were KrCl, XeBr and XeCl excilamps emitting maximum UV-radiation at 222, 283 and 308 nm, respectively. The parameters and choice of the lamps are discussed elsewhere (1). As the sample of humic substances, we used a Gumat-80 preparation (Gumat OOO, Irkutsk, Russia). The preparation was obtained by mechanochemical reaction of oxidized brown coal (Cheremkhovskoe field, Russia) with alkali (KOH, NaOH). The concentration of humic substances was selected so that the intensity of bioluminescence differed from the control by no more than 20%. The other samples of humic acids (HA) fractions were obtained from Fluka Chemical Co and prepared from peat of Tomsk region.

We have shown that efficient detoxification of an alcoholic solution of investigated furocoumarins (synthesized in Taras Shevchenko Kiev National University, Ukraine) occurs as a result of addition of humic substances. We have observed a dependence of the detoxification both on the structure of the studied compounds and on the characteristics of the source. After photoexcitation the bond lengths in the furocoumarins change and the humic substances detoxify the irradiated solution more efficiently. The degradation of herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) in water by the combination process of UV-irradiation, humic acids and activated sludge treatment has been studied. The photoreaction rate of all irradiated samples was lowest for the sample irradiated at 308 nm (the XeCl excilamp) in the absence and in the presence of humic acids, and highest for the sample irradiated at 222 nm (the KrCl excilamp). HAs apparently catalyzed the formation some different not toxic photoproducts of 2,4-D after UV-irradiation treatment by excilamps.

Acknowledgements

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Stabilization of Iron (II) Species in Humic Based Nanocomposite as Shown by XAFS and Mössbauer Spectroscopy

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Iron is a mineral nutrient presenting in plants and animals, it is crucial to living beings because it takes part in electron transporting and biosynthesis processes in cells. Iron deficiency is the most common nutrient deficiency in the world. Since iron can mainly be obtained from the foods, it is vital to consume iron rich meat and vegetables so that the body can absorb the iron from the diet. Bioavailibility of iron forms Fe (II) is higher than Fe (III). Humic substances are perspective matrix for bringing active compounds in living systems. Thus to obtain humic based samples containing Fe (II) is an issue of the day.

Goal of this research was to synthesis humic-based compounds containing stable iron (II) and to characterize redox speciation and local neighborhood of Fe atoms using XAFS and Mössbauer spectroscopy. Iron humate was obtained from commercially available potassium humate and iron (II) sulfate at pH 10 with stirring under pH control. Iron content was determined using spectrophotometer in the form of complex with o-phenantroline after oxidative digestion. Total iron content was 11 mass%. Results of EXAFS analysis and first shell approximation are given in the Figure 1.

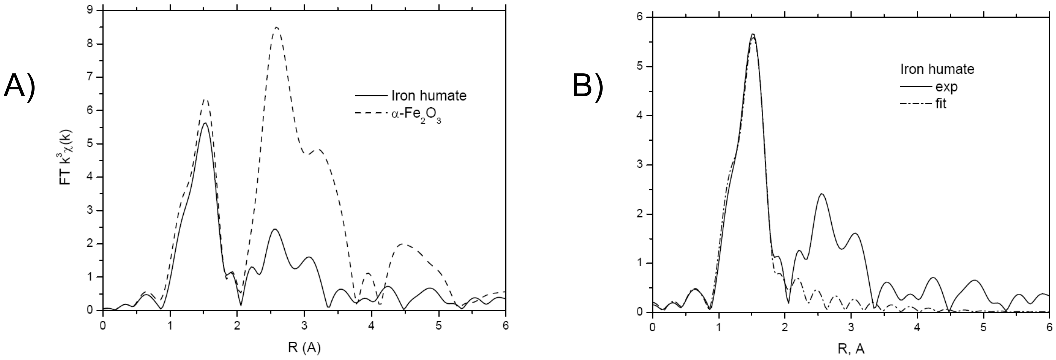

Figure 1. FT of EXAFS spectra of iron humate in comparison with standard α-Fe_2O_3 (A) and first shell approximation of local neighborhood of Fe (B).

The parameters of the first shell were R_{Fe-O} 1.975±0.015Å, N_O 4.8±0.9, σ^2_O 0.011±0.002, E_O -3.1±2.3 with fit quality R_f 0.65%. Results of EXAFS have shown that iron is surrounded with oxygen atoms in slightly distorted octahedral coordination and has Fe-O distance and structure similar to iron (III) oxide. No traces of iron (II) was found using X-ray adsorption spectroscopy. Redox speciation of iron was characterized using Mössbauer spectroscopy; parameters are given in the Table 1.

Table 1. Mössbauer parameters of iron humate at 5 K

Iron species	δ, mm/s	Δ, mm/s	H, kOe	S,%	G, mm/s
Fe (III)	0.46	-0.07	481	50	1.04
	0.43	-0.11	412	23	1.14
	0.51	0.65	-	12	0.70
Fe (II)	1.26	2.74	-	16	0.47

As shown by Mössbauer spectroscopy iron in the sample is presented with Fe (III) and Fe (II), about 75 % of total iron consist of superparamagnetic iron (III) oxides nanoparticles and about 16 % of total iron is stabilized in the form of Fe (II) which is expected to be bioavailable for plants and animals.

We acknowledge the Russian Found of Small Business Support (UMNIK 2010 project) and RFBR (project #09-02-12257-ofi_m) for financial support.

The mechanisms of regulatory influence of biologically active substances of humic nature on the organism of animals

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Use of humic substances in the ration of a highly productive nature of the bird provides an increase in the number of biological products and safety of animals. Proved that humic preparations from peat show no toxic and embryotoxic action, they do not accumulate in the organism, is not teratogenic. In the organism of animal preparations of humic nature are involved in the metabolism with the formation of end products. There are different views on the mechanism of action of humic substances in the organism of animals. These hypotheses are mainly associated with certain biological properties of humic substances such as the ability to influence the state of biological membranes and their permeability to different substrates, direct participation in the metabolic and bioenergetic reactions, hormone like on the structural and functional relationships. However, the mechanism of action of preparation on the humic nature of the animal organism is not fully clarified. It gives the opportunity to make hypothesis that could explain the involvement of these substances in the regulatory processes that activate the synthesis of biological production in the organism of highly productive poultry. In our opinion, the nature of humic substances when released into the gastro-intestinal canal are partially split in different compartments of the digestive system with the participation of digestive enzymes. In this case, both nuclear and peripheral part of a complex heterocyclic molecules of humic compounds are active. The experiments proved that the chicken and laying hens increased activity of hydrolytic enzymes not only in the chyme, but in the shell of small intestine mucosal ulcers, as well as in pancreatic tissue. As a result, the intestine is a change of regulatory programs at the expense of both the humic substances and their fragments, as well as the hydrolysis products of feed components. Simultaneously with these processes in the morpho-functional structure of the liver activates intracellular peptidhydrolas with redistribution of their localization in subcellular structures. This in turn is reflected in a change program of the enzyme-inhibitor and enzyme-activator interactions with the participation of the adenylylcyclase system and changes in calcium levels. Due to the influence of the identified regulatory mechanisms for the implementation of genetic information in the liver activates the synthesis of proteins of blood. In this case, increases in serum total protein, albumin and globulin fractions and the individual proteins (fibronectin, etc.). Of feedback additional information molecules in serum provides a new level of homeostasis, which corresponds to the higher productivity of the birds. In addition, in highly productive poultry under the influence of biologically active substances of humic nature is increased nonspecific resistance and immune responsiveness.

Criteria of the biological activity estimation of the new immunomodulative humic preparations

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In connection with polyfunctionality of humic substances, occurrence of new preparations of the humic nature and expansion of spheres of their application in medicine and veterinary science the necessity of unification, perfection of the existing and creations of the new methods of an estimation of their biological activity arises.

In Research laboratory on humic substances. named by. Prof. L.A.Christeva, created in the Dnepropetrovsk state agrouniversity still half a century ago, the scrining system of an estimation of biological activity of humic preparations and raw materials for their manufacture is developed and introduced. However creation of new preparations of the directed action, in particular, immunomodulative, has demanded working out of new criteria for comparison purposes their efficiency. Therefore in laboratory the scheme of a three-stage estimation of biological activity of such preparations is developed. At the first step the characteristic of physical and chemical properties and a chemical spectrum of operating substances (humic acids, microcells, pH, etc.) is spent. On the second degree the general estimation of biological activity with application of the scrining systems and also an estimation of potential toxicity and biosafety of preparations is spent. At the third step it is investigated immunomodulative effects of the preparations, with the use of the complex of tests according to influence on indicators of specific immunity of an animal, and nonspecific resistance.

The received results testify that immunomodulative humic preparations different in technology of receptions, differ on degree of influence on indicators of the humoral and cellular immunity, specific and nonspecific resistance that allows to apply them differentially depending on a condition of immune system.

Comparative Sensitivity of Standard Test–Organisms to Commercial Humates of Different Origin

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The effects of HS on living organisms are in a great measure uniformly beneficial as has been shown for plants, microorganisms, yeasts, fishes, birds, warm-blooded animals and humans. Therefore commercial humates (HUM) produced by industrial companies are promoted as fodder additives for livestock, poultry, in aquaculture and for ornamental fish and plants. The variety of organic sources and technologies of manufacturing provide differences in properties and activity of HUM. One of the most important parameters in HUM' quality is the potential for the material to be ecotoxic. The objectives of the study were i) to compare the sensitivity of standard test-systems to humates produced from different raw materials, ii) to select the most appropriate method for HUM-biotesting and iii) to reveal the influence of the molecular parameters of HUM on the test-reactions of organisms from different trophical levels and taxonomic groups. The solutions of 5 HUM products, manufactured from coalified and plant materials, have been tested at the concentrations of 0.005 – 2 g l^{-1}. The bioassays were carried out using a set of biotest-systems, including luminescent bacteria (Biotox system) and soil microbial community, protozoa (*Paramecium caudatum*), microalgae (*Cllorella vulgaris*), higher plants (*Raddish sp*. and *Sinapis alba*), and *in vitro* sperm cell tests of warm-blooded animals.

The assays of the acute toxicity in test-systems demonstrated that different biosensors have unequal responses to HUM:

a) the culture of cells protozoan *P. caudatum* as well as culture of animal cells in vitro did not reveal any negative reactions to HUM at any of the concentrations tested. The survival and mobility of these cells in the control sample without HUM and in the presence of any kind of humates did not decrease;

b) the luminescence in the Biotox system was depressed under the influence of HUM manufactured from coalfield materials. At the same time HUM originated from plant material in stimulated the luminescence of bacteria at all concentrations tested. Application of community level physiological profiling approach (multisubstrate testing) for soil microbial community allowed to reveal differences in functional biodiversity due to HUM effect;

c) the plant-biotest systems (the microalgae and the higher plants) demonstrated different sensitivity to coal and plant-origin humates. The latter stimulated plant growth at certain periods of plant development, whereas samples of coal HUM at concentrations of 1-2 g l^{-1} depressed plant development.

Thus, responses of standard test-organisms to the investigated HUM products varied greatly. Some test-systems demonstrated different response on HUM produced from coalified materials with high content of humic acids and HUM from peat containing more fulvics. Using the community level physiological profiling approaches (multisubstrate testing) was showed to be promising to estimate influence of HUM on microbial communities affected. Mostly the toxicity level of examined HUM depended on their origin that is closely related to their chemical structure.

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Combination of high resolution mass-spectrometry (FT-ICR) and nuclear magnetic resonance (NMR) for analysis of mumijo (shilajit)

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Native Mumijo is a blackish-brown exudation, of variable consistencies, obtained from steep rocks of different formations found in the Himalayas at altitudes between 1000-5000 m, from Arunachal Pradesh in the East, to Kashmir in the West. Mumijo also is found in other mountain ranges of the world, e.g. Afganisthan (Hindukush, Badakh-Shan), Australia (Northern Pollock Ranges), and in the former USSR (Tien-Shan, Pamir, Caucasus, Ural). There are several regional synonyms for mumijo, the second-most common one being Shilajit (in Sanskrit “destroyer of weakness”) [1]

Currently available methods based on mass spectrometric study of complex mixtures (crude oil, humic substances) allow to characterize the sample by determining the molecular formulas for mixture components based on exact mass measurement. [2]. However, this analysis will be more effective if we supplement the mass-spectrometric data with quantitative and structural information obtained by NMR [2].

Altai mumijo sample in the form of food supplement “Purified Altaj mumijo” produced by Company “Evalar” was studied. In-house software «Transhumus» was used for data processing.

In addition to brutto formulas of the components of the sample, FT-ICR mass spectrometry allows to obtain information on regular structures and building blocks of the mixture by the mass difference statistics analysis. Comparison of this structural information with quantitative NMR structural information may be used to validate the semi-quantitative data from FT-ICR spectra, and also to assess the ionization efficiency of different components of the mixture.

Results:

1) Part of total ion current corresponding to ions of NMR structural units series and corresponding to structural units series for mass differences statistic was determined. Abundance of structural groups discovered by NMR and by mass difference statistics was compared

2) NMR structural groups and component formulas from mass-spectrometric data were used to construct a graph of structure-group relationships for components of the complex mixture. Connectivity degree of the graph was estimated, and possible explanation of observed graph structure was given.

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Synthesis of Adhesive Silanol Derivatives and Their Sorption onto Mineral Support

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The goals of this study were twofold: first, humic materials were modified to acquire a novel, tailored property not inherent within natural humics (mineral-adhesive ability); and, second, humic derivatives were immobilized onto mineral support to obtain humic biocompatible nanocoating. To introduce new function in native humic materials, alkoxy-silylation was used.

The commercial potassium humate (Sakhalin Humate, Biomir 2000 Ltd., Russia) (CHS) was used for all modifications. 3-amino-propyltriethoxy-silane (APTES) was used for treatment of CHS. The choice of APTES was provided by the presence of reactive amino groups in its structure which can yield amide bonds upon reaction with carboxyl and carbonyl groups. In addition, APTES is commercially available organosilane suitable for preparative production of the corresponding derivatives.

Silanol derivatives of CHS were obtained by its condensation with APTES. The reaction was run at five different APTES-to-humics ratios, nominally: 0.2, 0.3, 0.4, 0.5 and 1 g of APTES per g of CHS. These ratios corresponded to different extents of modification associated with carboxyl groups available within the humic backbone. Depending on modification degree, the corresponding samples were designated CHS-APTES-20, CHS-APTES-30, CHS-APTES-40, CHS-APTES-50, and CHS-APTES-100. Sorption of the modified HA was carried out on 100 mg of silica gel. The total volume of experimental solution was 10 mL, concentration of humic samples was set in the range from 0.1 to 4 g/L. All sorption experiments were conducted in phosphate buffer (0,03 M, pH 6.0). Equilibrium time was 24 hours.

Figure 1 shows sorption isotherms for the silanol derivatives samples with maximum and minimum modification degree on silica gel. It can be seen that the larger sorption was observed for the sample with maximum modification degree (CHS-APTS-100), which displayed the higer affinity for silica gel surface.

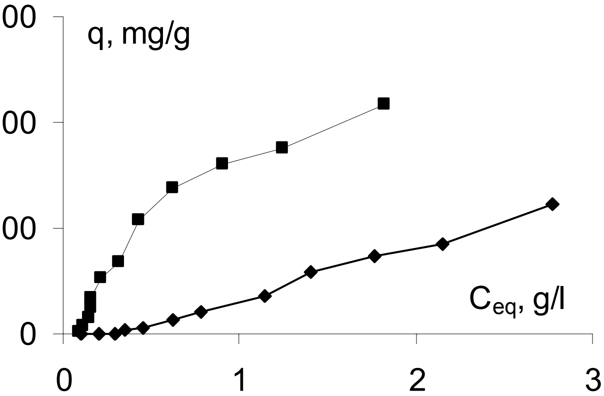

Figure 1. Sorption isotherms of the silanol derivatives of leonardite HA onto silica gel. ■ - CHS-APTES-100; ♦ - CHS-APTES-20

It was shown that all silanolized humic derivatives had high affinity for sorption on silica gel which are characterized with highly developed surface area. Maximum sorption achieved was 220 mg per gram of silica gel for CHS-APTES-100. The conclusion was made that the silanolized HS could be used for producing biocompatible coatings on mineral surfaces which can suit a whole suite of biomedical applications.

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Biological activity of humic lipids

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Lipid fractions of lucustrine deposits gave rise to a range of medicinal preparations, such as EPLIR, which combines antioxidant and hepatoprotective properties. In this connection, it is appealing to investigate composition and properties of peat lipids as new sources of natural biologically active substances, since their content in peats is dozens of times higher than that in lacustrine deposits. The results of investigations point to a large content of sterols, particularly β- sitosterol (to 6 - 7 %) in lipids of high-moor peats. Pigments are represented by carotinoids, chlorophylls *a* and *b*, and pheophytins. Vitamins, such as ascorbic and nicotinic acids, thiamine (B_1) and riboflavin (B_2) are also found in lipids, but in modest proportions.

The presence of polyphenol compounds (phenolcarboxylic acids, catechines), carotinoids, and aminoacids in lipids, compounds with a functional group containing a mobile hydrogen atom – OH, -NH, and –SH, controls their antioxidative activity (AOA). The amount of antioxidants (AO) in peat lipids is higher than that in lucustrine deposits and is found to be 0.35-1.3 mol/kg. Lipid fractions contain one or several AO types differing in their oxidation constants and rates, hence in reactivities. Natural AOs contained in peats inhibit peroxidation of lipids and prevent degradation of their bioactivity.

Sitosterols found in lipids are most highly bioactive substances among the above-mentioned groups. Sitosterol-containing preparations are used for treatment of benign prostatic hypertrophy, as they inhibit the synthesis of cyclooxygenase and leukotriene. β-sitosterol exhibiting an anticholesterolemic action is widely used in preparations for atherosclerosis. Given the fact that many of the diseases are caused by radical and oxidative factors, and lipids contain a large number of AOs, development of preparations inhibiting these factors is very important.

We have investigated antiinflammatory and hepatoprotective activity of lipids of high-moor peat, its fractions - polysaccharides and polyphenols, as well as combinations of peat lipids and Eplir in the ratio 1:1 and 2:1.

The experiments were performed on Vistar rats weighing 180-220 g. Use was made of experimental models of carrageenin-induced inflammation and acute toxic hepatitis in rats caused by administration of carbon tetrachloride. The dose of preparations was 30 mg/kg.

As a result of these studies it was found out that all investigated preparations exhibited anti-inflammatory and hepatoprotective activity. The degree of suppression of a carrageenin-induced lung edema was as high as 21 – 41% compared to untreated animals. The combination of peat lipids and eplir in the ratio 1:1 exhibited a more pronounced anti-inflammatory effect.

The preparations under study also showed a hepatoprotective effect. Hepatoprotective therapy was followed by a regression of biochemical disorders provoked by tetrachlormethane. The activity of blood ferments in the rats reduced 1.3-2.3 times. The concentration of total and bound bilirubin decreased 1.2-1.9 times. It was found out that the highest therapeutic effect in the case of acute toxic hepatitis was achieved by the mixture of peat lipids and eplir in the ratio 1:1.

Thus, as a result of treatment of carrageenin-induced inflammation and acute toxic hepatitis, the investigated lipids of peat, its fractions - peat polysaccharides and polyphenols, as well as combinations of peat lipids and Eplir in the ratio 1:1 and 2:1 were found to possess apparent therapeutic properties.

Viscous properties of the solutions of copolymers used in the synthesis of polymerporphyrins for medical applications

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Nowadays the synthesis of polymers for medical and biologic purposes is one of the actual trends in the chemistry of high molecular compounds. Modified polymers containing porphyrin derivatives in lateral branches are among them. They are characterized by unique biological, photochemical and catalytic activity and are used in phothdynamic therapy of cancer.

Synthesis of polymerporphyrins from macromolecular syntons consists of a number of consecutive polymer similar transformations carried out in solutions. That is why the properties of solutions, the parameters of polymer-solvent interactions, the "quality" of a solvent are of great importance for handling the reactions and for the achievement of needed characteristics of the end products.

Copolymers of styrene (St) and methylacrylate (MA) were studied. They were synthesized using the suspension method at the following ratios (St : MA) – 80:20, 70:30, 50:50, 30:70, 20:80 (% by weight). The values of characteristic viscosity [η] and Huggins constant K_x for the solutions of the given copolymers in toluene and ДMFA at various temperatures were defined. It was shown that the solutions of the studied copolymers in toluene belong to the systems with low critical temperature of dissolution, whereas the solutions in DMFA – to the systems with top critical temperature of dissolution (Fig.1). Thus, the expressed influence of solvent nature on the properties of copolymer solutions was revealed.

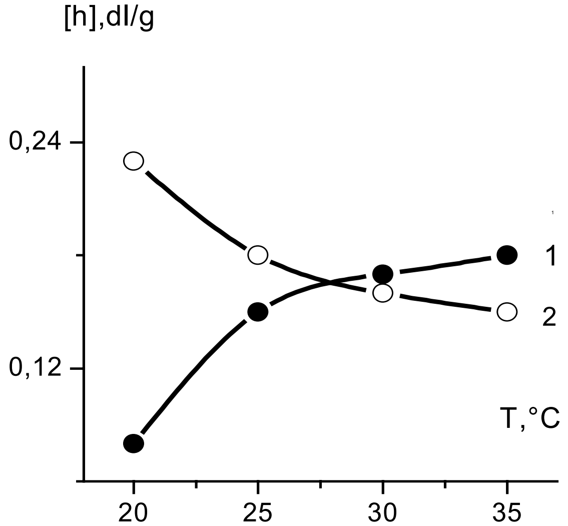

Figure 1. Characteristic viscosity of copolymer St : MA (80:20 % by weight) as the function of solution temperature. Solvents: 1 – DMFA, 2 – toluene.

The initial St and MA copolymers were further modified via reduction by lithium aluminum hydride. As the result, threefold copolymers St: MA: allylic alcohol and St: allylic alcohol copolymers were obtained. Using the method of capillary viscometry, the influence of ether and hydroxyl groups content in copolymers on viscometric properties of their solutions ([η], K_x and root-mean-square distance between the ends of circuits) was accessed at temperatures between 20^0C and 35^0C.

Chemotherapeutic effect of humic substances low mineralized silt sulphide muds on pathogenic and opportunistic microorganisms

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Objective: Study of the influence of the drugs peloid humic series and their derivatives on pathogenic and opportunistic microorganisms, as a promising basis for the development of chemotherapeutic drugs.

Materials and methods: We investigated the various fractions of humic substances low mineralized silt sulphide muds (peloids) lake Molochka sanatorium "Sergievsk mineral water": hymatomelanic [HMA], fulvic [FA], humic [HA] and humus [HsA] acid (concentrations 0,25%, 0,1%, 0,01%). We investigated the chelate complexes of humic peloids substances with ions of mercury (II), silver ions, iron ions (II), zinc ions in the same concentrations. The test-cultures of microorganisms were used to determine the antimicrobial activity of substances: 1) Protococcoid algae – *Scenedesmus quadricauda*; 2) Gram-negative bacteria – *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853); 3) Gram-positive bacteria – *Staphylococcus aureus* (ATCC 25293), *Bacillus subtilis* (ATCC 6633); 4) Yeast-like fungi – *Candida utilis* (LIA-01); 5) Mycobacterium – *Mycobacterium tuberculosis* (H37Rv), *Mycobacterium tuberculosis* (highly antibiotic-resistant strain isolated from patients). We Used culture media for microbial growth: Lowenstein-Jensen medium for culture of *Mycobacterium*; Prat's medium for culture of *Scenedesmus quadricauda;* Mueller-Hinton agar for culture Gram-negative and Gram-positive bacteria, Yeast-like fungi.

The active component of the derivatives of humic substances is a metal cation. The humic components options: 1. Increased penentrability through biological membranes; 2. Masking bactericidal component; 3.Tropism drug to microorganisms; 4. Reducing the toxic effect of metals on macro-organisms.

Results: All peloids preparations accelerate the growth of *Sc. quadricauda*. Effect was observed in direct proportion to the concentration in comparison with control samples.

Hymatomelanic, fulvic, humic, humus acid peloids have no cytolytic activity against the studied microorganisms. But often a static activity to an increase in *E. coli, Ps. aeruginosa* – all studied Gram-negative microorganisms. Stimulate the growth of the investigated Gram-positive microorganisms – *St. aureus, B. subtilis and M. tuberculosis* with respect to the control. Also show cytolytic activity to the growth of *C. albicans* at concentrations of less 0.1%, and no effect at concentrations more 0.1%.

All investigated chelate complexes of humic substances with ions of mercury (II), silver ions, iron ions (II), zinc ions in all investigated concentrations exhibit cytolytic activity against the test-cultures of microorganisms.

The lowest antimicrobial activity among the studied peloids preparations showed fulvic acid chelate complexes with ions of zinc and iron (II) – a zone of suppression of microbial growth less than 9 mm. The greatest antimicrobial effect of chelate complexes have 0,25% of humic substances: *E. coli* (zone growth suppression 20±0,1mm) – HsA^*Hg^{2+}; *Ps. aeruginosa* (20±0,1mm) – HA^*Ag^{+}; *St. aureus* (24±0,1mm) – HA^*Hg^{2+}; *B. subtilis* (16±0,1mm) – chelate complexes of humic substances with Ag^{+}; *C. utilis* (25±0,1mm) – HA^*Ag^{+}; *M. tuberculosis* – HA^*Hg^{2+} and HA^*Ag^{+}.

Conclusions: Humic substances low mineralized silt sulphide muds are the optimal components for further elaboration on their basis of the selective and differential-diagnostic nutrient medium for the mycobacteria and other pathogenic and opportunistic microorganisms. Chemotherapeutic study of chelate complexes of humic peloids substances characterize them as a promising substance for further development based on these broad-spectrum antimicrobial action, including anti-TB drugs of natural origin.

Хитозан и его производные в качестве новых адьювантов для вакцинирования

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Хитозан, получаемый деацетилированием природного полисахарида - хитина, представляет собой линейный сополимер, содержащий блоки мономерных звеньев глюкозамина и N-ацетилглюкозамина. Высокоочищенный хитозан малотоксичен, быстро биодеградирует под действием ферментов, находящихся внутри различных тканей и клеток организма. Олигомеры, образующиеся в ходе деполимеризации, оказывают многообразное стимулирующее действие на ряд важных процессов в организме.

В последние десятилетия хитозан, справедливо названный «веществом 21-го века», находит все большее и большее применение в медицине, сельском хозяйстве, биотехнологии, и ряде других областей науки и техники. Настоящее сообщение будет посвящено некоторым аспектам биологического действия хитозана на иммунную систему животных.

Наиболее эффективные современные вакцины, как правило, содержат так называемые адьюванты, роль которых заключается в стимуляции ряда процессов, приводящих к усилению иммунного ответа. Однако, используемые в настоящее время адьюванты обладают рядом негативных свойств: заметной токсичностью, медленной биодеградируемостью и медленным выведением из организма. Их применение может приводить в ряде случаев к чрезмерной активации системы врожденного иммунитета и, тем самым, наносить серьезный вред вакцинируемому организму. Поэтому проблема создания высокоэффективных и максимально безопасных адьювантов для вакцинирования остается по-прежнему актуальной.

Экспериментами, проведенными коллективом сотрудников московского НИИ вакцин и сывороток РАМН показано, что добавление раствора глутамата хитозония, а также микро-наночастиц некоторых солей хитозония в состав инактивированных гриппозных вакцин, вводимых парентерально, увеличивает титры антител при однократной иммунизации в 4 – 5 раз, а при двукратной иммунизации - .в 6 – 10 раз (в сравнении с титрами антител при иммунизации без хитозана). Кроме того, было обнаружено, что соли хитозония (СХ) способствуют образованию антител к дрейфовым вариантам вируса гриппа.

После вакцинации мышей инактивированным вирусом гриппа A/H5N2 и 0,5%-ным раствором СХ или 0,5%-ной суспензией СХ в виде микро-наночастиц защитный эффект при последующем заражении мышей живым вирусом гриппа возрастал в сотни раз по сравнению с мышами, вакцинированными без производных хитозана.

Высокая эффективность СХ в качестве адьюванта была продемонстрирована и при вакцинировании животных инактивированной полиомиелитной вакциной.

Было изучено влияние различных форм СХ и/или комплекса СХ с инактивированной гриппозной вакциной на синтез ряда цитокинов и интерлейкинов, а также на пролиферацию клеток иммунной системы, экспрессию ряда Toll-like рецепторов, синтез гамма-интерферона, TNF-альфа и др. факторов, участвующих в стимуляции системы иммунитета.

Сформулирована гипотеза, согласно которой производные хитозана не только активируют систему врожденного иммунитета, но также способствуют более эффективной презентации антигенов за счет образования межмолекулярного комплекса поликатионных участков хитозония с молекулами антигенов с одновременным экспонированием N-ацетилглюкозаминовых лигандов к эндоцитозным рецепторам (в частности маннозным рецепторам) на поверхности антигенпрезентирующих клеток.

Макромолекулярные модуляторы действия лекарственных препаратов

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Синтетические амфифильные блок-сополимеры в последние годы находят все более широкое применение в медицине и фармакологии в качестве носителей для доставки лекарств, эмульгаторов или усилителей иммунного ответа . Исследования последних лет показали, что многие амфифильные соболимеры могут быть полезны для решения актуальной проблемы современной онкологии - преодоления устойчивости раковых клеток к широкому кругу лекарственных препаратов. Это явление, получившее название множественной лекарственной устойчивости, возникает в процессе химиотерапии и представляет собой серьезное препятствие для успешного лечения онкологических больных. Оказалось, что плюроники способны восстанавливать чувствительность таких устойчивых клеток к противоопухолевым антибиотикам, а иногда даже делают их более чувствительными по сравнению с исходными опухолевыми клетками. Лекарственный препарат, содержащий плюроники и доксорубицин (SP1049c), в настоящее время проходит клинические испытания за рубежом. Тем не менее, в литературе практически отсутствуют экспериментальные данные о взаимосвязи между структурой амфифильных молекул и их способностью вызывать возмущения в упаковке липидного бислоя.

В настоящей работе исследовано более 30 электронейтральных амфифильных сополимеров, различающихся по химической природе. Гидрофобные блоки сополимеров были представлены полипропиленоксидом, полидиметилсилоксаном или углеводородными радикалами, в качестве гидрофильной составляющей в сополимеры входили полиэтиленоксид, декстран или полиглицерин. По макромолекулярной архитектуре исследованные сополимеры относились к линейным (двух- или трехблочным), звездообразным или гребнеобразным структурам.

Изучение взаимодействия сополимеров с модельными и биологическими мембранами показало, что встраивание сополимеров в мембраны приводит к увеличению их проницаемости и ускорению флип-флопа. Уменьшение гидрофильной части сополимеров и увеличение размеров их гидрофобной части приводит к усилению их воздействия на структуру липидного бислоя. При этом макромолекулярная архитектура полимера во многом определяет характер его влияния на барьерные свойства липидных мембран. Блочная архитектрура макромолекулы полимера в большей степени способствует увеличению его способности возмущать липидные мембраны без формирования трансмембранных гидрофильных пор. При этом такие полимеры вызывают ускорение флип-флопа липидов и транспорта незаряженных соединений через бислой. Сополимеры, имеющие структуру молекулярных щеток, т.е. способные к многоточечным взаимодействиям с мембраной, имеют ярко выраженную склонность к формированию гидрофильных пор в липидной мембране. Показано, что способность сополимеров к изменению свойств модельных липидных мембран коррелирует с их воздействием на накопление противоопухолевого антибиотика доксорубицина в раковых клетках, проявляющих множественную устойчивость к действию лекарств.

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Оптимизация способов получения полимерных композиционных материалов на основе препреговой технологии для протезно-ортопедической техники

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В настоящее время в лечении ортопедических заболеваний используется новое поколение конструкционных материалов – полимерные композиционные материалы (ПКМ), армированные фиброволокнами. Наиболее предпочтительной и обеспечивающей высокое, стабильное качество является технология формования изделий из препрегов ПКМ, которые позволили принципиально изменить методику консервативного лечения и реабилитацию травматологических и ортопедических больных в послеоперационном периоде. Препрег, готовый для переработки продукт (полуфабрикат) предварительной пропитки термореактивным связующим упрочняющих материалов тканой и нетканой структуры, применяемый в производстве изделий из армированных полимерных композиционных материалов. Технология изготовления из препрегов ПКМ позволяет хорошо адаптировать их по контурам конечности и, таким образом, расширить показания для применения на различных стадиях лечения переломов и позволяет вернуть конечности утерянную функцию.

В производстве ортезов в настоящее время используются препреги с упрочняющими материалами на основе карбоновых, стеклянных, натуральных и синтетических (арамидных, лавсановых, нейлоновых и т.п.) волокон. В качестве связующего, как правило, используются различные модификации эпоксидных смол, позволяющих вести формование и полимеризацию композиционного материала при достаточно низких температурах (120-130°С) и достаточно низком избыточном давлении – под вакуумом, что расширяет спектр применяемых материалов, в том числе и вспомогательных, используемых при формовании изделий, упрощает технологию.

ПКМ армированные фиброволокнами имеют уникальные, по сравнению с традиционными материалами, массовые и механические характеристики. В ФГУП «ЦИТО» Минздравсоцразвития России под руководством Г.Н. Булгакова проводятся работы по оптимизации препреговой технологии для получения ортезов из карбоновых тканей. При вакуумной технологии формования препрегов существенно возрастает точность моделирования внутренней поверхности ортеза, за счет плотного облегания материалом гипсового слепка. Это в свою очередь повышает требования к более высокой точности обработки позитива и подготовки его для создания такого ортеза. Применяемая технология изготовления в полной мере отвечает основным требованиям, предъявляемым к ортопедическим изделиям: точная пригонка; простота конструкции; прочность и легкость.

Использование армированных фиброволокнами композиционных материалов - препрегов предоставляет широкие возможности конструирования высокотехнологичных протезно-ортопедических изделий по показателям жестко-эластичности конструкции без использования металлических шин. За счет комбинирования той или другой форм структуры упрочняющих материалов с различными толщинами нитей возможно получение необходимых прочностных и упругих характеристик в различных элементах и участках каркасов ортеза, что в свою очередь обеспечивает стабильное удержание сегментов конечности в заданном положении за счет заложенной упругости всей системы и жесткого крепления шарниров в ортезе.

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Разработана, внедряется и продолжает развиваться технология АВЗ – антистрессовое высокоурожайное земледелие и технология ОЖЗ – экологического органического живого земледелия для возделывания с/х культур: от зерновых, картофеля, технических культур до овощных, ягодных, плодовых и декоративных культур.

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«Гуми, Гуми-М» – универсальные, антистрессовые, ростоускоряющие, иммуностимулирующие, биоактивированные по молекулярному весу и набору микроэлементов гуминовые удобрения. Используются для замачивания семян, а также при обработке вегетирующих растений в смеси с гербицидами, инсектицидами или без них с целью ускорения ростовых процессов и повышения устойчивости ко всем стрессовым факторам, в том числе и гербицидам.

«Гуми-Богатый» – высокоэффективное комплексное NPK – гуминовое удобрение с содержанием основных макро- и микроэлементов в хелатной форме и Фитоспорин-М.

«**Фитоспорин-М»** – универсальный промышленный биофунгицид пролонгированного действия и длительного срока хранения усилен препаратом Гуми. Предназначен для защиты сельскохозяйственных культур от грибных и бактериальных заболеваний. Обладает высокой фунгицидной и росторегулирующей активностью, действует сразу после обработки семян и вегетирующих растений. Совместим с химическими пестицидами. Экологичен, безвреден для человека, животных, птиц, насекомых.

«Бионекс-Кеми» основное – биоактивированное азотно-фосфорное- калийное удобрение с микроэлементами в полимерно-хелатной форме с природным биофунгицидом Фитоспорином-М и эликсиром плодородия Гуми.

«Бионекс-Кеми» водорастворимое – полностью растворимое биоактивированное NPK удобрение с микроэлементами и природным биофунгицидом Фитоспорином-М. Применяется для повышения урожайности в качестве легкоусвояемых подкормок для всех с/х культур.

«Гуми-90 Хозяин плодородия» – органическое гумусное удобрение для восстановления гумуса и плодородия почвы.

Все препараты являются биогенными стимуляторами природного происхождения, не содержат ГМО-продуктов и гормонов.

Ветеринарный препарат **«Микровитам-комплекс»** – оптимально сбалансированный комплекс, содержащий аминокислоты, витамины и микроэлементы. Предназначен для восполнения потребности организма в аминокислотах, витаминах, микроэлементах, для улучшения обменных процессов, повышения стрессоустойчивости и резистентности организма животных к инфекционным заболеваниям.

Ветеринарный микробиологический препарат **«Споровит-порошок»** – препарат-пробиотик, предназначен для профилактики и лечения широкого спектра инфекционных заболеваний, укрепления иммунитета, улучшения обменных процессов в организме.

Кормовая добавка **«Биогумитель»** – комплекс гуминовых веществ, аминокислот, микроэлементов и микробиологических БАВ с пробиотической активностью. Обладает антиоксидантными, антибактериальными, противовирусными свойствами, повышает неспецифическую резистентность организма и иммунитет, регулирует обмена веществ, повышают активность пищеварительных и внутриклеточных ферментативных систем.

Ряд других биоактивированных препаратов находятся в научных и экспериментальных разработках.

Center of Innovation Biotechnology and Pharmaceutics “Park of Active Molecules” (CIBPh “PAM”)

Rakhimdzhan Roziev
“Medbiopharm” Ltd., Obninsk, Russia

медбиофарм
ИННОВАЦИОННАЯ
КОМПАНИЯ

The business idea of CIBPh PAM is to growth the innovation product ready-to-operate.

As contrasted with existing technology transferanters, business-incubators, centers of collective access, we will not grow an entrepreneur from a research-idea carrier. Idea carrier will be a partner of the proposed Center and, if he want, will appear as scientific advisor in the project work.

CIBPh after subject expertise performed by its own expenses will solve the tasks of investment drawing and deal structuring, provide full-scope support and realization of the project, contract manufacturing organization, market promotion.

Using corresponding engineering and technical infrastructure, professional skills and competences (both own and partners’) CIBPh is the manager, coordinator and main executor of projects, three-in-one. Each function is separate, self-sufficient business unit of the whole process.

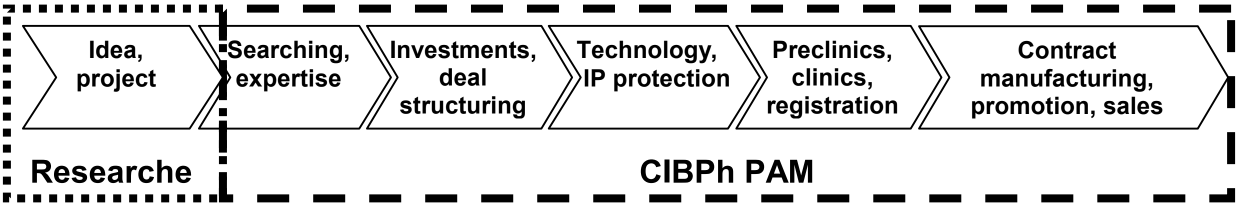

Figure 1. CIBPh in the process of project realization

So, the basis of proposed business idea is the creation of the center of competence and full-scope support of an idea to a market.

As the result of business idea realization, new innovation pharmaceutical, diagnostic, health food products will be launched into a market.

Also, new services demanded on the market of development and realization of innovation projects will be provided. Today such services are required by RUSNANO, RVC and other innovation oriented companies.

These services include: expertise, licensing, required trials performing, registration, pilot production, small product etc.

Also, services for development and selection of method of nanostructurisation of active molecules will be demanded. This market seems to be very perspective, as it provides the lowering of therapeutic dose keeping high activity, and increasing of selectivity and new pharmacological properties appearance, such as water solubility of oil soluble substances, expanding of shelf life, more convenient form of release.

Non-Commercial Partnership “Center for Biogenic Resources “Humus Sapiens”

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Non-commercial Partnership “Center for Biogenic Resources “Humus Sapiens” (NCP CBR “Humus Sapiens” was founded in 2007 by three leading Russian institutions in the field of chemistry, petroleum chemistry and chemical engineering: Lomonosov Moscow State University, Mendeleev Russian Chemical Technology University, and Gubkin Russian State University of Oil and Gas. The goal of NCP CBR “Humus Sapiens” is to provide research and development support to the “green chemistry” industry in Russia which implies a use of plant and humic materials as feedstock and plasticizes environmentally friendly technologies. The products of “green chemistry” include biofuel, biofertilizers, food additives, bioplastics, nanomaterials, composites and other

To reach these goals the NCP CBR “Humus Sapiens” concentrate its efforts on the following activities:

gives expert-analytical conclusions on innovative potential of “green chemistry” products and technologies;

launches and performs research and development projects in the field of “green chemistry”;

creates data bases on humic and plant raw materials and feedstock for “green chemistry”, on commercial products, producers and scientific centers active in this field;

develop and realizes standardization systems for humic-based and other bioproducts including development of reference materials and standard protocols;

organizes conferences for discussion of theoretic and applied problems in humic science and technology;

performs editorial and publishing activities for publishing scientific materials, conference papers and others;

forms the positive image of “green chemistry” products from humic and plant raw materials.

The portfolio of “Humus Sapiens” includes “know-how” for estimating quality and origin of commercial humic preparations by means of classification analysis based on date base containing data on more than 300 humic preparations. We also have two patented technologies for producing labeled humic preparation and for synthesis of silicon-containing self-adhesive preparations. Many more technologies are under development.

Некоммерческое партнерство «Экспертно-аналитический центр по проблемам органогенного сырья «Гумус Сапиенс»

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Некоммерческое партнерство «Экспертно-аналитический центр по проблемам органогенного сырья «Гумус Сапиенс» (НП ЭАЦПОС «Гумус Сапиенс») создано в 2007 г. Его учредителями стали лидирующие научно-образовательные учреждения России в области химии, нефтехимии и химической технологии: МГУ имени М.В. Ломоносова, РХТУ имени Д.И. Менделеева и РГУ нефти и газа имени И.М. Губкина. Цель партнерства – содействие становлению индустрии «зеленой химии» в России, которая предусматривает преимущественное использование в качестве сырья растительных и гуминовых ресурсов и реализует безотходные и экологически безопасные технологии. Продукты «зеленой химии» на основе растительного и гуминового сырья включают в себя биоэнергоносители, биоудобрения, наноматериалы, композиты, биопластики и другие инновационные продукты.

Для достижения поставленных целей НП ЭАЦПОС «Гумус Сапиенс» концентрирует свои усилия на следующих направлениях:

дает экспертно-аналитические заключения по оценке инновационного потенциала продуктов «зеленой химии» и соответствующих технологий;

выполняет научно-исследовательские и конструкторские разработки по приоритетным направлениям НП ЭАЦПОС «Гумус Сапиенс», формирует инновационные бизнес-проекты;

формирует базы данных по сырьевым ресурсам, коммерческим продуктам, поставщикам научных услуг, работающим в области органогенного сырья; организует информационно-консультационную деятельность;

разрабатывает и внедряет системы стандартизации продуктов на основе гуминового и растительного сырья, включая разработку стандартных образцов и стандартных методик;

созывает научные конференции и совещания для обсуждения теоретических и прикладных проблем гуминовой отрасли;

осуществляет издательскую деятельность, издает труды ученых, научных организаций, конференций и др.;

формирует позитивный имидж продуктов на основе органогенного сырья путем просветительской деятельности, в том числе с применением интернет-технологий.

На настоящий момент в портфеле инновационных разработок НП ЭАЦПОС «Гумус Сапиенс» способ оценки качества коммерческих гуматов и гуминоподобных соединений путем классификационного анализа, основанного на обширной базе данных (более трехсот препаратов) по структуре и свойствам природных и модифицированных гуминовых веществ, а также две патентованные технологии: получение меченых гуминовых препаратов и синтез кремнийсодержащих гуминовых веществ. Большое количество разработок завершено на лабораторном уровне и готово к масштабированию.