Carbon nanotube-based separation and analysis of nucleic acids

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Carbon nanotubes (CNTs) are graphite-like nano-sized substance showing unique structural / physicochemical properties and compatibility with biomolecules and living cells. The properties are attractive for the development of customizable materials and devices for biomedical applications [1]. Here, we present the combined application of CNTs as a sorbent and sensor component for the separation and analysis of nucleic acids.

To remove impurities and increase hydrophilicity, CVD-synthesized multi-walled CNTs were chemically oxidized under ultrasonic agitation. The procedure allowed controllable introduction of carboxyl groups onto CNTs to obtain stable water suspension of CNTs. Pre-oxidized CNTs were cast on the surface of disk or planar electrodes to form modified nanostructured layer. Electrode modification with CNTs considerably promotes the oxidation of nucleic acids and its constituents, possessing low electrochemical activity, owing to high effective area of CNTs and their electrocatalytic effect [2]. Redox center in nucleic acids is guanine nucleotide oxidized at about +1 V vs. Ag/AgCl to produce measurable current. The signal strongly depends on conformation and structural integrity of double-stranded DNAs. Particularly, DNA denaturation or strand cleavage are accompanied with an increase of the signal as a result of improvement of DNA adsorption on CNT-modified electrodes [3]. Using these electrodes several analytical methods for the evaluation of DNA structure integrity and detection of relevant DNA damages, including depurination and 8-oxoguanune formation, have been developed [3, 4].

Sensitivity of CNT-based sensors towards conformational changes in DNA is due to strong adsorption of DNA nitrogen bases on the surface of CNTs [5]. We studied the adsorption of several DNA forms on CNTs in solution using gel electrophoresis. We found that pre-oxidized CNTs preferentially bind damaged DNAs, e.g. denatured ds-DNA or restriction fragments of plasmid DNA. In the presence of Mg^{2+} and other divalent metal ions CNTs effectively bind supercoiled plasmid DNA due to chelate complex formation. The results presented form the basis for CNT-based analysis and separation of nucleic acids for diagnostics as well as therapy applications.

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Spontaneous haemoglobin Fe(II) oxidation in fullerene C_{60} water dispersion

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The effect of unmodified fullerenes, namely their anti(pro)oxidative activity, over the physiological function of protein molecules remains the subject of extensive research as the C60 can generate active oxygen forms by energy transfer or a C60 anion radical by electron transfer. Oxygenated form of haemoglobin Fe(II) is capable of spontaneous oxidation into the physiologically inactive form Fe(III).

In the experiments involving haemoglobin in fullerene water dispersion we have studied the effect of hydrated fullerene nanoclusters over the state of haemoglobin oxidation. Kinetics of spontaneous oxidation reaction of haemoglobin Fe(II) have been observed at 45°C and fullerene concentration 1 to 15 μg/ml within 5.2 - 7.9 pH range using UV-visible spectrometry.

The overall amount of oxygenated haemoglobin Fe(III) has been shown to rise considerably in presence of fullerene as compared to that in control protein solution. The effect increases both with the fullerene concentration and pH. Kinetic curves appeared to be essentially parallel suggesting almost no dependence of haemoglobin oxidation kinetics and rate of oxidation on the fullerene concentration. The dependence on pH can be explained by the protonation of distal histidine in haemoglobin molecule at low pH facilitating the haemoglobin oxidation. In that case fullerene contribution to the oxidation is of a less degree.

The results obtained indicate the prooxidative activity of fullerene water dispersion and suggest that fullerene most probably interacts with haemoglobin as an electron acceptor.

Bulk biocompatible composite nanomaterial

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Carbon nanotubes (CNT) possess mechanical properties (hardness H_v and breaking strength σ) close to the properties of diamond. Therefore, it should be expected that in a biological composite material, the additives of CNT will considerably increase its mechanical parameters H_v and σ.

We investigated the mechanical properties of the bulk composite nanomaterial made of bovine serum albumin (BSA) and additives of CNT, i.e. BSA+CNT. The samples were prepared in a chamber with controllable pressure, in which 25% water solution BSA+0.1-0.4 CNT (single-layered CNT of the NanoCarbLab firm) was subjected to the effect of laser irradiation with a wavelength of 970 nm and power of 8-20 W. In the technological mode, we varied the power and time of irradiation, CNT concentration, time and temperature of drying of samples varied.

The obtained nanomaterial BSA+CNT was black-colored with a glossy surface. Its consistence varied from soft rubber-like to solid depending on the parameters of the technological mode of preparation.

The investigated nanomaterial BSA+CNT had density ρ=1200-1250 kg/m^3, H_v=120-350 MPa, σ=20-40 MPa. Their ρ was by 10-15% above and H_v larger by a factor of 2-6 with respect to crystal albumin. The reached maximal mechanical parameters H_v and σ are comparable with parameters of the known materials, for example, organic glass (H_v=120-250 MPa), aluminum (H_v~200 MPa, σ~100 MPa), cast iron (H_v~500 MPa, σ=180-230 MPa), and human bone tissue (H_v~500 MPa, σ~15-50 MPa).

The above-described composite nanomaterial BSA+CNT can be used in the structure of various biocompatible and bioactive implants of the bone and cartilage tissues.

Research of durability of seams of the cartilage tissue with composite nanomaterial in the laser solder

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Carbon nanotubes (CNT) look like the most suitable component material of bulk compositions that can be promising in biomedical applications. The creation of the effective solder for welding the biological tissues with laser radiation is especially topical.

The breaking strength of laser seams of cartilage tissues with the application of the solders containing CNT is investigated. Earlier, the solders were applied to laser welding the biological tissues on the basis of water solutions of albumin with an absorber of laser radiation cardiogreen ICG [1]. We used solders on the basis of water solutions of bovine serum albumin with polymethine dyes PK7208 and PK 7213 and CNT of the NanoCarbLab firm.

As a result of measurements of the breaking strength of laser seams of the cartilage tissues of the bull and mutton tracheas, it is established that the application of solders with the CNT impurity increases the breaking strength σ of welded seams by a factor of 4-10 with respect to conventional compositions of solders [1]. The maximal σ~2-4 MPa for a seam were 8-12% of σ for the monolithic cartilage tissue and multiply surpassed the value σ=0.09 MPa with the solder on the basis of a water solution of albumin on fresh samples of a biological tissues of a pig in work [2].

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Antipodal effects of fullerene nanoparticles on biological tissues are determined by deformation hardening or softening

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Scaling of deformation stresses from atomic (<1 nm) to nanometer (< 100 nm) and global scale lengths shows that the micromechanisms of plastic deformation under all tests are the same for all the materials. This means that antipodal reactions (mechanical hardening WH, or softening, S) are of the same origin. The growing physical and chemical activity of nanoparticles in matrix concerns with their plastic size-effect and different functions of nanoparticles and mean-size particles in plastic WH, S (or dislocation-like cross slip) at low and moderate temperatures.

Although the carbon materials are very close in size and compatible with biological cells, BC, the fullerene nanoparticles have antipodal effects on BC. These effects depend on the structure, size, concentration of carbon particles and the mechanical properties, scale length, type (what part of the body and the place on evolution tree of the species), age and physiological status of BC. For example, literature data demonstrate that the fullerene concentration in water $(5\text{-}8)\cdot10^{-7}$ makes the high brain (the soft BC) disorders in fishes and the deaths of less differentiated and the softest BC of little water insects. In the same time the higher concentrations $(7\cdot10^{-6}\text{-}7\cdot10^{-5})$ mg/ ml of it increase the activity of brains in rats. Ultramicroscopy gives the direct evidence of BC deformation under the carbon nanoparticles, and this interaction brings the local inflammation and even peroxide oxidation of cell-membrane lipids, POL (oxidation stress of BC). These effects are not found in rigid tissues of fish gills and liver, but there are some microscale inflammations in liver cells and the activation of few genes on molecular scale only.

Fullerenes and bioions: ensembles of structures for nanobio

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Fullerenes C_{60} can interact with almost every biological molecules such as carbohydrates, proteins, bioregulative and DNA molecules. During investigations of these systems only simple two or three component models were constructed: C_{60} + biomolecule + water . In fact, real biological ensemble must to include one common component – bioions.

The aim of the presented work is to examine fullerene C_{60} abilities to bind with bioions. By using methods of molecular and quantium mechanics the spatial architecture and behaviour of ensembles consisting of C60 and bioions was investigated. It has been demonstrated that fullerenes have some interaction with single, pair and ensemble of bioions.

Understanding of mechanisms of interaction fullerenes with the integral component of real biological systems bioions is connected with finding-out of mechanisms of biological action of fullerenes.

At enough of ions in system it will be organised in such a manner that molecules of C_{60} is surrounded by cylinders formed by ions and are connected among themselves by chains of ions. Such structures are an example of self-organised nanosystems.

It has been shown that the investigated complexes will be steady in water to the environment, so other possibility of use of such complexes is creation of water-soluble fullerenes.

Conclusions:

- Fullerenes C60 can form complex bioions.
- On the basis of complexes fullerenes and bioions have been received self-assembled systems
- Creation water-soluble fullerenes on the basis of the investigated complexes is possible

Investigation of specific adsorption properties of silica gel in respect to blood plasma lipoproteides in the presence of fullerene

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In the present-day therapy, plasmasorption hold a separate position among methods of elimination of atherogenic lipoproteides from human blood. This method requires effective bio-compatible sorbents allowing selectively to eliminate atherogenic lipoproteides, retaining concurrently the other vitally important components. Here, in the present research, we have to do with adsorbtion characteristics of silica gel in respect to atherogenic lipoproteides (low-density lipoproteides), including modification of porous matrix with fullerene C_{60}. The samples of three sorts were studied: 1 – initial silica gel (porous diameter 2500 nm, specific surface 20m^2/g; 2 – prepared by dry mixing of fullerene and silica gel in vacuum; 3 – prepared by impregnation of silica with fullerene in ortho-dichlorbenzene solution.

The blood composition was determined as ratio of principal blood plasma components before and after contact with each of the samples. It was revealed following: all three silica gels did not have considerable adsorption capacitance in respect to so vitally important components as protein and triglyceride (concentration changes <8%); each of silica gels manifested itself as a rather effective adsorbent of atherogenic lipoproteides, but at that were not greatly differ in the elimination coefficient (20-30%); when used samples 2 and 3 (with fullerene), high-density lipoproteides were retained in the blood plasma (that is importantly), whereas the initial silica gel adsorbed this component essentially. The last circumstance shows preferences of the silica gels modified with fullerene before initial one.

The analogical set of measurements was undertaken after treatment of the samples in the conditions similar to standard sterilization (water, 100°C). It was appeared, that adsorption characteristics of silica gels with fullerene were not changed under treatment. Contrary, for the initial silica gel it was revealed an essential increase of the elimination coefficient in respect to high-density lipoproteides.

For the samples 1 and 2 NMR spectra at ^{1}H, ^{13}C, and ^{29}Si nuclei were measured.

Complexes of pristine fullerene C_{60} with proteins

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Previously we have showed the membranotropic mechanism of antiviral action of pristine fullerene C_{60}, which has become apparent only when water soluble preparations where molecules C_{60} are in low aggregated state e.g. C_{60}/PVP complex are used. In addition to this complex destroyed the influenza virions whereas the membranes of various cell lines were seemingly intact. This difference in action may be caused by the ability of isolated (monomeric) molecules of pristine fullerene to interact not only with lipids but also with proteins. Previously it was shown that molecules of pristine C_{60} can pass on from C_{60}/γ-cyclodextrin complex (1:2) to various proteins, e.g. to human serum albumin. We have showed that such transfer is also possible by using C_{60}/PVP complex. By this we have prepared various complexes of fullerene C_{60} with some proteins, e.g. bovine and human serum albumin, transthyretin etc.

The simple stirring of solutions of C_{60}/PVP complex and protein in PBS leads to formation of the C_{60}/protein adducts. The formation of these adducts can be easily detected by the appearance in the UV-VIS spectra absorption band of fullerene C_{60} at 330-335 nm in the protein-containing fraction. Despite of C_{60}/PVP complex, which is stable only in pure aqueous solutions, the obtained C_{60}/protein adducts are quite stable in various conditions and can be purified by ion exchange chromatography, gel filtration or by precipitation.

Electrophoresis data for transthyretin showed that binding with fullerene C_{60} did not cause the dissociation of its supramolecular complex to monomers. The influence of pristine fullerene C_{60} on the biological properties of proteins are now under investigation.