Posters

SPECTROSCOPIC STUDIES OF INTERACTIONS BETWEEN ORTHO DERIVATIVES OF P-DIMETHYLAMINOBENZOATE AND BOVINE SERUM ALBUMIN

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Understanding the interaction between organic molecule (potential drug) and the proteins is fundamentally essential, especially for medical diagnostics [1]. In this report, the interaction between bovine serum albumin (BSA) and two ortho derivatives of *p*-methylaminobenozate (methyl *o*-methoxy *p*-methylaminobenzoate (**I**) and methyl *o*-hydroxy *p*-methylaminobenzoate (**II**)) have been studied using steady-state spectroscopic technique. The molecule **I** dissolved in aprotic solvent exhibits only locally excited fluorescence, whereas the molecule **II** exhibits dual fluorescence i.e., emission form the locally excited state and the intramolecular proton transfer state [2]. In the first step of our studies, spectroscopic measurements were employed to investigate the nature of interactions of three biochemically important aromatic amino acids residues viz., tryptophan, tyrosine and phenylalanine (which are constituents of protein) with studied dyes [3-6]. The presence of isosbestic point in absorption and fluorescence spectra of **II** obtained in phosphate buffer, in the presence of tryptophan at its various concentrations, suggests the formation of 1:1 complex between molecule **II** and tryptophan. Similarly, **II** was found to strongly interact (specifically and universally) also with proteins (potential drug related with bovine serum albumin) by fluorescence quenching. The quenching mechanism between **I** and **II** bovine serum albumin was determined as mainly dynamic quenching, combined with static quenching.

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PRIMARY REACTIONS IN BACTERIORHODOPSIN PHOTOCYCLE – REVISITED

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Bacteriorodopsin (BR) is a protein and retinal complex found in purple membranes (PM) that acts as a light-driven proton pump. Under the influence of BR lighting, it is subject to cyclic reactions. It is generally accepted that the primary reaction (the first step of the photocycle), as a result of which energy is accumulated for further transformation of BR is the trans-cis isomerisation of the chromophore taking place without the "communication" of the chromophore with its immediate environment. There are suggestions, however, that another process (for example, the redistribution of electric charge along the chromophore) is the first step in the transformation of BR and that the closest surroundings of the chromophore, e.g. water molecules, can influence this step.

In order to explain both controversial issues, femtosecond absorption spectroscopy was applied and three types of samples were used: native PM, PM with fluorinated bacteriorhodopsin and PM deposited electrophoretically on SnO_2. The water content in the samples was regulated by reducing the pressure in a special cryostat. Because "dry" samples can be easily destroyed by irradiation with laser radiation, a special, very precise device was constructed that moved the cryostat with the sample in x-y direction.

It was noted that the kinetics and yields of femtosecond changes of native and fluorinated BR are different. The changes were strongly dependent on the water content in BP. The obtained results suggest that the redistribution of charges along the chromophore is a step earlier than its trans-cis isomerization. In addition, it can be stated (contrary to earlier publications) that the "communication" of the chromophore with the closest surroundings (eg. through water molecules) affects the original BR reactions. It is suggested that similar “electrostatic communication” between chromophore and opsin may take place in rhodopsins, visual complexes.

EVALUATION OF THE EFFECT OF ORGANOPHOSPHORUS FLAME RETARDANTS ON HUMAN ERYTHROCYTES

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Intensive growth of manufacturing of synthetic polymers present in our life increases risk of fire. That is why various methods are used in order to reduce flammability of daily use products. One of them is the usage of flame retardants, which are designed to slow down the combustion process, and thus affect the emission of smoke. This group of chemical compounds includes organophosphorus flame retardants. So far, there is insufficient data for evaluation of the toxic effects of these chemicals on the environment and living organisms.

The aim of this study was to determine hemolytic and oxidative properties of two selected phosphorus flame retardants – tris(2-chloroethyl) phosphate, and (2-chloroisopropyl) phosphate. The study assessed changes in cell viability and morphology (flow cytometric analysis of cell size and granulation) as well as alterations in methemoglobin and reactive oxygen species (ROS) levels in human erythrocytes. The erythrocytes were separated from blood (leucocyte-buffy coat) from healthy donors. Blood was obtained from the Regional Blood Donation and Blood Treatment Center in Łódź.

Hemolysis and methemoglobin content showed a tendency to increase along with the increasing concentrations of the compounds studied. Similarly, the level of ROS determined on the basis of the dichlorofluorescein fluorescence raised along with the increasing concentrations of the substances studied, but it did not reach high value.

The results of this study have shown that organophosphorus flame retardants are characterized by relatively low toxicity in comparison to the most commonly used brominated flame retardants (BFRs), because the majority of changes have been observed only at their highest concentrations, which may penetrate into the human body as a result of acute poisoning. The lowest concentrations of the tested compounds did not cause any statistically significant changes in the parameters analyzed.

FAST FIELD-CYCLING NMR RELAXOMETRY CHARACTERIZATION OF HYDROCOLLOIDAL SYSTEMS

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Hydrocolloids are polymers of biological or synthetic origin with of a large number of hydroxyl groups, widely used in food processing technologies as gelling agents, thickeners or fat and saccharose replacers. Water binding affects texture and processing characteristics, which is why knowledge of the state of water in such biopolymer suspensions is essential to understand and predict their behaviour during production, storage and thermal processing. A useful technique to study the state of water in foods is nuclear magnetic resonance (NMR); the usual way of probing the dynamics using NMR is to examine relaxation at different temperatures and assume a function for the temperature dependence of the correlation times. However, in such a way large temperature range needs to be covered, which can be problematic in foods, as its structure and properties are temperature dependent. The alternative is to determine so-called spectral density function of the substance by measuring spin-lattice relaxation time, T_1, over a wide range of Larmor frequencies. By using this so-called field-cycling (FC) technique one can probe the dynamical processes in the system [1].

The aim of the study was to acquire Nuclear Magnetic Relaxation Dispersion (NMRD) profiles of several binary systems based on agar, gelatin and carrageenan varying in concentration and temperature. Relaxation data complemented with viscosimetry measurements allowed to draw basic conclusions on the dynamics of water present in the systems and proved a potential of FC NMR relaxometry as tool to characterize food products.

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THE ROLE OF PICEATANNOL IN COUNTERACTING GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE AGGREGATION AND NUCLEAR TRANSLOCATION IN HIPPOCAMPAL CELLS

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The primary aim of modern neurobiology/science is to prevent or slow down the progression of neurodegenerative diseases. One available solution is supplementation with superfoods. To widen the knowledge

about compounds that are contained in various fruits and vegetables, we examined one naturally occurring stilbene derivative - piceatannol and its effect on glyceraldehyde-3-phosphate dehydrogenase (GAPDH). This enzyme is one of the most susceptible to oxidative modifications. Further, GAPDH changes, under certain conditions, promote and accelerate neurodegenerative processes [1]. In this study, we demonstrated how piceatannol influences on these processes.

The objective of the presented study was to determine whether piceatannol inhibits unfavourbale GAPDH nuclear translocation in hippocampal cells as well as protein aggregation induced by excessive oxidative stress. For this purpose we applied following methods:
MTT assay (cell viability), immunostaining and confocal microscopy, immunoprecipitation and Western Blot and flow cytometry analysis.

We found that piceatannol significantly suppresses GAPDH nuclear translocation as well as protein aggregation induced by excessive stress.

The piceatannol anti-aggregation activity and ability to counteract GAPDH nuclear translocation place this compound as a new drug candidate for *in vivo* tests.

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EFFECT OF CARDIOPROTECTIVE FLAVONOIDS ON THE ACTIVITY OF THE MITOCHONDRIAL BK_{Ca} CHANNEL

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Flavonoids belong to a large group of polyphenolic compounds that are widely present in plants. Some of them, including luteolin, quercetin or cyanidin, have been shown to be cardioprotective. Although the antioxidant effect of flavonoids has been long thought to be a crucial factor accounting for cellular cardioprotection [1,2]. Also, mitochondrial pathways (including mitochondrial large-conductance Ca^{2+}-regulated (mitoBK_{Ca} channel) are presently emerging potential targets for a specific pharmacological action of flavonoids in the anti-ischemic strategies [3].

The aim of these studies is the characterization of interactions between cardioprotective flavonoids and the mitoBK_{Ca} channel present in the inner mitochondrial membrane of the endothelial cells.

Single channel activity of the mitoBK_{Ca} was measured with patch-clamp of the mitoplasts isolated from endothelial cells (EA.hy926). Application of 3 μM cyanidin has an inhibitory effect. In the presence of luteolin, changes of open probability of the mitoBK_{Ca} channel were not observed. Furthermore, regulation of the mitoBK_{Ca} channel by flavonoids were studied in the presence of 0.5 mM dithiothreitol. Changes in the redox state causes that luteolin and cyanidin have activatory properties. Open probability of the mitoBK_{Ca} channel increase from 0.02 to 0.36 at -40 mV in the presence 10 uM cyanidin. However, quercetin has strong activating properties both under control conditions and reduced by DTT. Additionally, possible cytoprotective properties of quercetin with using apoptosis/necrosis assays were also studied.

We expect that our studies describing the regulation of mitochondrial potassium channels by the natural substances of plant origin will bring us closer to a better understanding of flavonoid-induced cytoprotective mechanisms.

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BIOLOGICAL PROPERTIES OF CHITOSAN-GRAPHENE NANOCOMPOSITES

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Chitosan is an amino-carbohydrate obtained from incomplete deacetylation of chitin. It is biocompatible, fully degradable, water-soluble and can be used as a colloidal solution, handled as a solvogel, triggered as a pH-responsive physical or chemical hydrogel, cast as thinner or thicker films, and shaped as self-standing microspheres to provide highly porous CO_2-dried monolithic aerogels or lyophilized cryogel scaffolds. These features account for implementing chitosan scaffolds in various fields, including scavenging

chemicals, tissue-engineering, wound-dressing, drug-release and food-packaging.

Graphene oxide is an increasingly studied nanomaterial that has recently been used as nanosized filler to build novel exfoliated nanocomposites. However, few functionalized graphene (oxide) derivatives are known, and informative studies dealing with the biological effects of graphene surface functionalization are currently missing in the open literature.

The aim of the study was to evaluate the effect of chitosan-graphene nanocomposites on human erythrocytes and hemoglobin.

Results shows the hemolytic activity after incubation time of 1, 3 and 24 h. All chitosan-reinforced graphene nanocomposite films induced hemolysis. After incubation for 1 and 3 h, the hemolysis of erythrocytes was approximately 6.5% with no statistically significant differences between composites. After 24 h of incubation, the changes are not statistically significant compared to the hemolysis obtained after shorter incubation times. As hemolysis was not dependent on incubation time, we investigated possible hemoglobin adsorption on the surface of chitosan-reinforced graphene films. After 3 h incubation of hemolysate with graphene composites, a negligible adsorption of hemoglobin was experienced. However, hemoglobin adsorption reached 22-29% after 24 h of incubation. These results suggest that hemoglobin released from erythrocytes remains adsorbed to chitosan-graphene films after 24 h, which causes a decrease in the hemoglobin content in the solution and was misread as a lack of hemolysis increase after 24 h incubation. Thus, the percentage of hemolysis after 24 h does not reflect real hemolytic activity but is rather associated with the accumulation of hemoglobin (released from erythrocytes) on the surface of graphene composites.

All chitosan-graphene films caused the oxidation of hemoglobin after 3 h of incubation with the erythrocytes. For the control, the percentage of methemoglobin after 3 h of incubation was only 1.8%, and after 24 h, the percentage increased to 4%. Statistically significant changes in the percentage of met-Hb content were observed for all graphene composites after 3 and 24 h incubation.

MOLECULAR MECHANISMS OF PHOTOPROTECTION IN THE PHOTOSYNTHETIC APPARATUS OF PLANTS

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Life on Earth is powered by the energy of light reaching our planet from the Sun, but utilization of this energy by living organisms is only possible thanks to the process of photosynthesis that converts the energy of electromagnetic radiation to the forms which can be directly used to drive biochemical reactions [1]. Photosynthesis in plants operates under conditions characterized by severe risk factors associated with the exposure to high light. Under excess light, violaxanthin (Vio) is converted rapidly to zeaxanthin (Zea), and this reaction is reversed under low light levels. Efficient and safe operation of the photosynthetic reactions is vital to plants and is assured by the activity of numerous regulatory processes functioning to increase excitations under low light and to quench excessive, potentially harmful excitations, under high light conditions. For many decades there has been a debate on the role of zeaxanthin, synthesized in the xanthophyll cycle, in photoprotective excitation quenching and conclusions from various studies are often contradictory [2,3].

Molecular spectroscopy techniques such as steady-state, time-resolved fluorescence and resonance Raman scattering were used in this work. Action of the xanthophyll cycle and chlorophyll excitation quenching were analyzed in *Arabidopsis thaliana*, the wild-type and two mutants, npq1 (lacking Vio de-epoxidase) and npq4 (lacking the PsbS protein demonstrated to be essential for an efficient Zea-dependent photoprotective excitation quenching). The results of the experiments show that zeaxanthin can account for ca. 50 % of the photoprotective quenching of chlorophyll excitations.

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INVESTIGATION OF DRUGS MOLECULES RELEASE FROM POLYURETHANE HYDROGELS CONTAINING CLAY NANOPARTICLES

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Polyurethane hydrogels due to their unique swelling properties are very versatile in case of possible applications in many fields, especially in biomedicine [1]. The ability to maintain a hydrated environment, high capacity to absorb the solution and the ability of the polymer to release active substances made them good candidates for biomedical applications [2]. These features enable the design of a moist hydrogel dressing to facilitate wound healing as well as relieve pain, releasing the drug

into the skin [3]. Improvement of material properties is possible by adding nanoparticles that expand the intermolecular spaces in the polyurethane matrix and increase the swelling capacity of the polymer matrix [4].

The description of swelling and release of active substances is crucial aspect examined in terms of the applicability of hydrogel materials. The transport of solutes in swollen gel membranes is subject to two mechanisms: dissolved substances penetrate the membrane through the pores filled with solvent (diffusion) and the reaction of the polymer to the stresses exerted by the attack of solvent molecules occurs (relaxation) [5].

The main purpose of our research is to achieved material with predetermined and well defined hardness, elasticity, and with appropriate swelling and release profiles. In previous research we described method of synthesis and studies of basic mechanical properties and structural properties [6-8].

In the present studies, we examined polyurethane nanocomposite hydrogels doped with various amount of and nanofiller – Cloisite® 30B. In particular, we investigated the influence of Cloisite® 30B on the swelling and release of active substances: naproxen sodium and paracetamol. The presence of clay mineral plates in hydrogels remarkably improves the swelling capability, but on the other hand slows down the release. We also performed an accurate theoretical analysis in different theoretical and semi-empirical models [8-9].

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ONE-TRYPTOPHAN MUTANTS AS MARKERS OF TRIMERIC MAMMALIAN PURINE NUCLEOSIDE PHOSPHORYLASE UNFOLDING

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The folding and unfolding of oligomeric protein are not well explored. To this group belongs homotrimeric purine nucleoside phosphorylase (PNP) – the enzyme that plays a key role in the nucleoside and nucleotide metabolic salvage pathway, and is a target for anti-cancer and immune system suppressing therapies [1]. Our studies have shown that although the enzyme exists in a trimeric form, each subunit functions independently [2], and monomers, if exist, are unstable and prone to aggregation [3].

To answer the question how the unfolding of PNP proceeds – during one step, without presence of monomers, or in two steps where trimer first dissociates to unstable monomers, three one-tryptophan mutants were obtained (W16-PNP, W94-PNP and W178-PNP). All these mutants have catalytic properties similar to that of the wild type PNP. Their fluorescence spectra show a clear difference between folded and unfolded forms making them a good tool for characterizing PNP folding/unfolding processes.

The stopped-flow unfolding measurements initialized by mixing of folded protein with buffer containing high concentration of denaturant - guanidinium hydrochloride show that the tryptophan environment changes the fastest for the W94-PNP mutant, in which Trp is located closest to the symmetry axis of the protein. It suggests that during unfolding, PNP trimer first dissociates into unstable monomers.

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CHARACTERISTIC OF SPECTROSCOPIC PROPERTIES AND ANTIOXIDANT ACTIVITY OF NEW SYNTHESIZED ALPHA-TOCOPHEROL DERIVATIVE

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This work concerns spectroscopic, DLS and antioxidant studies of alpha-tocopherol (Toc) analog modified at the O-1 position, named 1-carba-alpha-tocopherol (1CT). The studied vitamin E derivative contains the 1,2,3,4-tetrahydronaphthalene skeleton instead of the chroman ring. This modification should significantly change its physico-chemical and spectroscopic properties compared to the parent tocopherol.

In this study, spectroscopic properties (absorption, fluorescence and fluorescence lifetime) of 1CT in homogeneous environments and in liposomes composed of dipalmitoylphosphatidyl choline (DPPC) were measured. In order to estimate the influence of 1CT on the properties of model membranes dynamic light scattering (DLS) technique was used. For this derivative, antioxidative activity tests using the DPPH radicals were also performed.

In organic solvents with different physical properties, the absorption maxima for 1CT was located at similar positions (283-286nm), with extinction coefficients ranging from $1200M^{-1}cm^{-1}$ in octanol to $8000M^{-1}cm^{-1}$ in hexane. The investigated Toc analog exhibited a blue shift of 9-12nm compared to Toc. The fluorescence maximum of 1CT in the investigated solvents was found at the wavelengths ranging from 303 to 311nm, and is blue shifted at about 18nm compared to Toc.

In a model lipid membrane Toc exhibited emission spectra which consisted of an unstructured band with maximum at 325nm. The fluorescence maximum of 1CT in DPPC was found at 306nm and this position was held within a wide fluorophore concentration range. For 1CT the linear fluorescence increase was observed with increasing concentration of this derivative what suggests that the observed emission arises from a monomeric form of 1CT. In liposomes the emission maximum and fluorescence lifetime of Toc analog were similar to those observed in methanol, which suggests medium value of dielectric constant and low viscosity environment. Simultaneously, the fluorescence lifetime of 1CT (3,5ns) incorporated into DPPC is longer that observed for Toc (1,2ns).

The particle size distribution in the DPPC suspension was determined using DLS method. The mean values of liposome sizes (110nm) were determined from the analysis of number of peaks and was not changed significantly in the presence of different amounts of 1CT.

The antioxidant activity of 1CT was determined by the method of quenching DPPH radicals, which relies on measuring absorbance intensity at the characteristic for DPPH wavelength equal to 517nm. The antioxidant properties of 1CT was compared with that of the parent Toc sample. The obtained results confirmed that 1CT reveals antiradical properties and quenches DPPH radicals. However, its antioxidant efficiency was much lower that observed for free Toc. This phenomena results from deprivation of heterocyclic oxygen, which plays a key role in the antioxidant activity of vitamin E.

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COMPUTATIONAL STUDY OF SELECTED 4-HYDROXYMETHYL-3-AMINOACRIDINE DERIVATIVES WITH ANTICANCER ACTIVITY P

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4-hydroxymethyl-3-aminoacridine (4-HM-3-AA) derivatives were synthesized [1] and evaluated for anticancer activity [2] in laboratories in France. It is interesting that the most cytotoxic compounds (i) intercalate to DNA but do not inhibit DNA topoisomerases activity, and (ii) differ in cell distribution (Peixoto *et al.*, 2009). In this research the molecular properties of selected 4-HM-3-AA derivatives were studied using quantum mechanical calculations methods and then the results were discussed to explain the difference in their biological activity. It has been found that there are some differences in both structural parameters and electronic properties of molecules, which may explain their different biological behavior.

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The calculations were performed on a computer and software in the Laboratory of Computer and Analytical Techniques, University of Lodz, Poland.

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EXAMINATION OF THE CADMIUM-CHLOROPHYLL COMPLEX: SPECTRAL PROPERTIES, KINETIC AND REASONS FOR INHIBITING PHOTOSYNTHESIS

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Heavy metals can be taken up by plants from the environment and transported with water to stems and leaves [1] thus causing plant growth inhibition [2], formation of reactive oxygen species [3], and inhibition of photosynthesis [1]. In order to reveal the mechanism of cadmium-induced chlorophyll degradation, spectroscopic analyses were carried out, using a series of chlorophyll ($C=1x10^{-5}M$) solutions with $CdCl_2$ (from $C=1x10^{-5}M$ to $9x10^{-3}M$) in methanol. With increasing Cd^{2+} concentration, both, Q_y and the Soret chlorophyll bands were shifted by 9 nm towards the short-wave range. New absorption bands for the reaction products were formed at 656nm and 420nm. The fluorescence spectra were shifted hypsochromically by 11 nm (677 nm to 666 nm) relative to the chlorophyll fluorescence band. The final absorption and fluorescence spectra of the pure complex were recorded after 240h for the $C_{Cd}=1x10^{-5}M$ and for the $C_{Cd}=9x10^{-3}M$ after 17h.. The reaction rate constants were increased in samples from $k=1.510x10^{-5}M^{-1}min^{-1}$ for $C_{Cd}=1x10^{-5}M$ to $k=13.350x10^{-4}M^{-1}min^{-1}$ for $C_{Cd}=9x10^{-3}M$. The experiments show that cadmium is bound into the chlorophyll molecule substituting its magnesium. In plants intoxicated with cadmium, taken up from contaminated soil, the energy transfer between Chl and Cd-Chl will be impaired, which may be one of the reasons for the inhibition of photosynthesis. This is indicated by two times smaller overlap integrals of the Cd-Chl absorption spectrum with the Chl fluorescence spectrum, $I_{Chl,CdChl}=2.4223x10^{-13}cm^3/M$ (twice lower probability of transfer) in comparison with overlap integral for Chl→Chl transfer: $I_{Chl,Chl}=4.6210x10^{-13}cm^3/M$), and lower Förster critical distance for resonance energy transfer: $R_{oChl\rightarrow CdChl}=46.773$Å, $R_{oChl\rightarrow Chl}=52.086$Å.

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RESONANCE RAMAN SPECTROSCOPY STUDY ON LOCALIZATION AND ORIENTATION OF LUTEIN IN A LIPID BILAYER

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Lutein, together with zeaxanthin and meso-zeaxanthin, are xanthophyll pigments with special significance for humans. In the human body they accumulate selectively in the retina of the eye and thus protect the retina from damage [1]. These compounds are antioxidants scavenging efficiently free radicals and besides they act as a light filter absorbing harmful to the eye shortwave radiation, hence their presence in the yellow spot of the retina is essential for maintaining the proper functioning of the vision organ. However, they not only affect the eye, but also the various tissues of living organisms.

In biological membranes xanthophylls are present as components of lipid phase or in the form of a protein complex [2]. In the presented work, giant unilamellar vesicles (GUVs) were used as a model of biological membranes to verify the response of Raman spectroscopy to the interaction occurring between lutein and dipalmitoylphosphatidylcholine (DPPC). It seems to be helpful for understanding the processes taking place inside the living organisms.

Lutein-containing GUVs were formed at 0.5 mol % xanthophyll concentration with respect to DPPC lipid (Avanti Polar Lipids). Before liposomes preparation, crystalline lutein (Extrasynthese) was repurified by using HPLC technique and then has been added to a lipid solution in ethanol. Obtained mixture were deposited to two platinum electrodes fixed in the Teflon holder at a distance of 4 mm, placed for 1h in a vacuum (to remove organic residues) and next in a cuvette which contained the buffer solution (1.4 mL, 20 mM Tricine, 10 mM KCl, pH 7.6). Finally, electric connections were attached to the AC field supply (DF 1641A). Electroformation process was carried out over 2 h with an applied AC sinusoidal field with 10 Hz frequency and voltage 3 V (peak-to-peak). The temperature was stabilized at 45°C.

The obtained results confirm that application of the resonance Raman technique enables to determine the orientation of the transition dipoles of xanthophylls molecules due to the photoselection process. Analysis of Raman images of individual liposomes shows that lutein can adopt two orientations: perpendicular and parallel with respect to the membrane plane. In case of using the lowest possible laser power, the preferred molecules orientation is vertical and at these points, spectroscopic analysis indicates the presence of the trans-xanthophyll forms in the unilamellar vesicle. On the other hand, the increase in laser power causes the formation of more distorted structures of lutein, which are oriented horizontally in relation to the membrane plane (signal in the upper and lower sector of liposome).

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CHANGES IN ANTIOXIDANT ENZYMES ACTIVITIES AND REACTIVE OXYGEN SPECIES LEVEL IN HUMAN ERYTHROCYTHES EXPOSED TO SELECTED PHTHALATES

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Phthalates have been extensively used as plasticizers in various branches of industry including food, cosmetic and pharmaceutical. Phthalates do not form covalent bonds with other compounds, thus they can easily migrate from various products, and then reach the body with air, food and water. Significant concentrations of phthalates and their metabolites have been determined in urine, breast milk, blood serum, venous blood, and cord blood.

It has been shown that phthalates like di-n-butyl phthalate (DBP), butylbenzyl phthalate (BBP) as well as their metabolites including mono-n-butylphthalate (MBP) and mono-benzylphthalate (MBzP) can induce oxidative stress. Therefore, the aim of our work was to evaluate the effect of selected phthalates on the activities of antioxidant enzymes, i.e. superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) and the level of reactive oxygen species (ROS) in human erythrocytes.

The erythrocytes were incubated with the compounds studied in the concentrations ranging from 0.5 to 500 μg/ml for 24 h. It has been found that DBP, BBP and their metabolites: MBP, MBzP induced ROS (including $^{\bullet}OH$) formation, increased CAT activity and decreased the activities of SOD and GSH-Px.

It has been noted that the strongest alterations in ROS formation, and antioxidant enzymes activities were induced by DBP and BBP in the concentration of 2.5 μg/mL.

SPECTROSCOPY OF TRI-CYCLIC GUANINE AND ISOGUANINE DERIVATIVES AND THEIR RIBOSIDES

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Tri-cyclic analogs of natural purines and their derivatives are known to react with many enzymes of purine metabolism [1], and are important intermediates of the chemical mutagenesis.

The purine-nucleoside phosphorylase enzyme (PNP, E.C.2.4.2.1) is responsible for the regulation of the various nucleoside concentrations within the living cells, and a target of many types of pharmaceutical interventions [2]. PNP isolated from *E. coli* is active towards tri-cyclic εAdo and its 2-aza analog [3]. In the absence of phosphate ions, it is possible to observe the reverse reaction - attachment of the sugar moiety to the tri-cyclic base, where the second substrate is a phosphorylated sugar (α-D-ribose-1-phosphate, R1P).

Our investigations have shown that 1,N^2-ethenoguanine is an excellent substrate for PNP from *E. coli*, with catalytic and Michaelis' constants comparable to that for ribosylation of the parent guanine. The reverse reaction (phosphorolysis of the nucleoside) is also easily observed in the presence of phosphate ions. These facts may be important in view of a significant mutagenic role of 1,N^2-ethgenoguanine lesion in many organisms. The isomeric N^2,3-ethenoguanine is not a substrate for PNP form *E. coli* and calf spleen [4].

Spectrophotometric titrations of the 1,N^6-etheno-isoguanine (εisoGua) indicate that this compound exists as a neutral species at pH 4.5–7, and above pH 8 undergoes deprotonation. The anionic forms are virtually nonfluorescent, while the neutral form and the cation are strongly fluorescent, with maxima at ~400 nm.

Ribosylation of εisoGua, catalyzed by PNP from *E. coli* gave two products: The highly fluorescent N9-riboside, and N7-riboside with less intense fluorescence shifted to ~355 nm. The analogous reaction catalyzed by the calf PNP gave one main product, very intensely fluorescent, but with UV absorption spectrum markedly shifted to the longer wavelengths, identified as N^6-riboside. All ribosides may be useful as fluorescent probes in enzymology.

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STRUCTURAL CHANGES OF COAL CAUSED BY AUTOCHTHONIC MICROBIOTA - FTIR STUDIES

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The aim of the research was recognition of structural changes of coals as an effect of activity of autochthonous microorganisms. FTIR (Fourier Transformed Infrared Spectroscopy) was applied to analyze pristine samples of polish hard coals and lignites and same materials subjected to long-term anaerobic microcosm incubations. Microbial activity and community structure were studied using gas chromatography and next generation sequencing.

Stimulation of microbiota resulted in a decrease of free C=O (>1740cm^{-1}), probably as an effect of activity of species that utilize the Wood–Ljungdahl pathway which enable some anaerobic *Bacteria* and *Archaea* both energy and biomass production [1]. The surface area of the peak characteristic of a -COOH stretching vibration decreased upon incubation, indicating the possibility of usage of this group by the acetotrophic methanogens [2].

The lignites revealed a significant reorganization of the structure concerning aromatic/aliphatic character revealed by the change in the regions representing aromatic CHx stretching (3000-3100 cm^{-1}), aromatic C=C ring stretching (1550-1650 cm^{-1}), aromatics' CHx out of plane deformation (650-900 cm^{-1}), aliphatic CHx stretching (2800-3000 cm^{-1}) and aliphatic CHx bending (1300-1550cm^{-1}). When considering hard coals, in samples analyzed, the decrease in aromaticity was accompanied by an increase in aliphaticity and CH_2/CH_3 ratio. The released $-CH_3$ and $^{-}OCH_3$ groups comprise a readily available substrate for methylotrophic microorganisms.

In the microbiota composition of hard coals as well as lignites *Bacteria* comprised 97-99% of the community. Among them, the major phylum was *Proteobacteria* (43-61%). In the pristine communities *Archaea* constituted only 0.03-1.51% and increased several times during anaerobic incubation. The structural changes of lignites and hard coals indicate that these materials harbor microbial communities capable of anaerobic degradation of the organic matter and by providing fermentation substrates may support methanogenesis.

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REGULATION OF MITOCHONDRIAL POTASSIUM CHANNELS BY FLAVONOIDS P

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Rapid, electrogenic transport through cell membranes is mediated by many different types of potassium channels. Recently, many studies focus on the intracellular potassium transport. The protection of cardiac cells against ischemia/reperfusion injury by activators of the mitochondrial K_{ATP} channel and the mitochondrial BK_{Ca} channel is now widely accepted. Mitochondrial potassium transport-dependent cytoprotection against ischemia/reperfusion and oxidative stress induced injury has also been demonstrated in other numerous tissues [1].

In recent years, the subject of many studies are chemical compounds found in plants. Due to the numerous biological effects, a particularly interesting group are flavonoids. Interest in health benefits of flavonoids has increased due to their potent antioxidant and free-radical scavenging activities. The biological activity, bioavailability and low toxicity set broad prospects of the usage of some of these substances as potential therapeutics for a number of human diseases. Some flavonoids have also been shown to be cardioprotective. Although the antioxidant effect of flavonoids has been long thought to be a crucial factor accounting for cellular cardioprotection, mitochondrial pathways (including mitochondrial ion channels) are presently emerging potential targets for a specific pharmacological action of some flavonoids in the anti-ischemic strategies [2,3].

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THE INHIBITORY EFFECT OF STATINS ON VOLTAGE-GATED POTASSIUM CHANNELS Kv1.3 IN JURKAT T CELLS P

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Voltage-gated potassium channels of the Kv1.3 type are widely expressed in many cells, both normal and cancer. Kv1.3 channels participate in several processes including proliferation and apoptosis of Kv1.3-channels' expressing normal and cancer cells. It is known that some small-molecule organic inhibitors of the channels including biologically active plant-derived polycyclic compounds may selectively induce apoptosis of Kv1.3 channels' expressing cancer cells, while sparing normal ones. These compounds may be promising candidates for a putative application in a therapy of some cancer disorders, characterized by an over-expression of Kv1.3 channels, such as breast, colon and lymph node cancer, melanoma or B-type chronic lymphocytic leukaemia (B-CLL) [1].

Statins are compounds known as inhibitors of 3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase. This leads to an inhibition of biosynthesis of cholesterol and isoprenoid metabolites. Therefore, statins are widely applied in a treatment of hypercholesterolemia and atherosclerosis [2]. It was shown that statins – mevastatin and simvastatin exert antiproliferative, pro-apoptotic and reversing drug resistance effect in human colon adenocarcinoma cell line LoVo and its doxorubicin-resistant subline LoVo/Dx [2]. Studies performed in our electrophysiological laboratory applying the whole-cell patch-clamp technique showed that statins: mevastatin and simvastatin are effective inhibitors of Kv1.3 channels in cancer cells – human T cell line Jurkat. It was shown that an application of mevastatin and simvastatin in the concentration range from 7.5 μM to 30 μM inhibited the channels in a concentration-dependent manner. The inhibitory effect was partially reversible. The inhibition was accompanied by a significant acceleration of the currents' inactivation without any significant change of the activation rate. In the case of an application of another statin: pravastatin – an inhibitory effect on Kv1.3 channels was observed only at the concentration of 50 μM, whereas at lower concentrations no significant inhibition was observed. A mechanism of the channels' inhibition and its contribution to a regulation of cancer cells' proliferation and apoptosis by the statins is discussed.

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THE EFFECT OF BROMOPHENOLIC FLAME RETARDANTS ON DNA DAMAGE IN HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS P

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Bromophenolic flame retardants (BFRs) are synthetic substances widely used in the industry (manufacture of electrical and electronic equipment, textiles, furniture and other everyday products) [1,2]. Products containing BFRs protect property; however there are fears about harmful impact of these substances on human health.

In 2012, the European Food Safety Authority concluded that it is not possible to determine the health risk posed by BFRs due to insufficient data on the presence of these compounds in edibles and the food chain, and the negligible number of toxicological data.

Damage to DNA of human peripheral blood mononuclear cells (PBMCs) may contribute to impaired immune response, which may lead to autoimmune diseases or cancer development. That is why in this study, we have assessed the effect of selected BFRs, i.e. tetrabromobisphenol A (TBBPA), tetrabromobisphenol S (TBBPS), 2,4,6-tribromophenol (2,4,6-TBP) and pentabromo-phenol (PBP) on double-strand breaks creation and hydroxyl radical formation in human PBMCs.

The cells were incubated with the compounds studied in the concentrations ranging from 0.01 to 10 μg/ml for 1 or 24 h. DNA damage was assessed using neutral version of the comment assay [3], while hydroxyl radical formation was determined by flow cytometry using fluorescent probe – hydroxyphenyl fluorescein.

The results of this study have shown that TBBPA at 1 and 10 μg/ml caused statistically significant increase in DNA double strand-breaks (DSBs) formation, while other compounds studied did not induce DNA damage. It is well-known that highly reactive oxygen species (mainly hydroxyl radical) are involved in DSBs formation [4]. We have observed that only TBBPA at 1 and 10 μg/ml increased hydroxyl radical level in human PBMCs.

In conclusion, TBBPA caused low level of DNA damage in human PBMCS, which was mainly due to hydroxyl radical formation in this cell type.

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