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Posters

1. Antioxidative and sun protective properties of natural compounds applied in cosmetics

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A lot of kind sunscreens are available on the Polish market. The purpose of this study was the estimation of sunscreen physical parameters (antioxidative and sun protective properties) of natural compounds applied in photoprotective cosmetics. The sun protective parameters were investigated by UV-VIS spectroscopy. Thin layers of photoprotectants were subjected to spectral analysis. Physical photoprotectants are substances that completely reflect the sunlight. The light source used had spectral characteristics similar to that of the sunlight. A spectroscope detected the light spectrum of the beam passing across the sample. Measurements times were the same for all samples. Spectra were recorded using an optical spectrometer Ocean Optics USB 4000 in the 384–1020 nm band and cover glass with a homogeneous layer of the preparation. Considerable differences between analyzed samples were found.

Antioxidant potential of the tested compounds was studied with the aid of electron paramagnetic resonance spectroscopy at X-band microwave frequencies (9.3 GHz). After adding a standard solution of free radicals (DPPH) to analyzed preparations, the EPR spectra parameters were estimated. Amplitudes (A), linewidths (DB_{pp}) and integral intensities (I) of the spectra were determined. The Rapid Scan Unit of Jagmar Firm (Kraków, Poland) was used. The first derivative EPR curves were measured with the low microwave power of 2.2 mW to avoid he microwave saturation effects of the spectra. The integral intensities (I) of the EPR spectra were calculated by double integration of the first derivative curves.

2. Intermolecular interactions in the aggregated forms of carotenoid pigments

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Carotenoid pigments are commonly present in living cells and are recognized to play several vital physiological functions, including stabilization of the structure of proteins and biomembranes. Often, carotenoids form aggregated structures (e.g. in lipid membranes) and the role of such structures is not known. Intermolecular interactions responsible for the formation and stabilization of the aggregated forms of carotenoid pigments: β-carotene, zeaxanthin (3,3'-dihydroxy-β-carotene) and canthaxanthin (4,4'-dione-β-carotene) were investigated by means of resonance Raman spectroscopy and infrared absorption spectroscopy (FTIR). Aggregated forms of pigments have been obtained by evaporation from two kinds of solvents. The first group consisted of solvents which may contain trace amounts of water, while the second group consisted of solvents which did not contain water molecules. Analysis of the FTIR spectra shows that aggregated forms of all carotenoids examined, formed by evaporation from the hydrated solvents, bind water molecules in their structures, despite the fact that β-carotene lacks any polar groups which may be potentially involved in the hydrogen bond formation. Further analysis of the infrared absorption spectra shows that water molecules can form weak hydrogen bonds with C–H groups of the carotenoids. Resonance Raman scattering spectroscopy indicates that aggregated forms of carotenoids, formed without water are characterized by relatively strong van der Waals interactions, demonstrated by shift of the band attributed to the C=C stretching vibrations towards lower wavenumbers. A relatively high intensity of the band attributed to the =C–H out-of-plane deformations of carotenoids in aggregated forms binding water molecules indicates twisting of the polyene chain.

3. The effect of K^+ and Na^+ ions on the aggregation of antibiotic amphotericin B in the lipid membranes

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The amphiphilic polyene antibiotic amphotericin B (AmB) is the drug of choice in the treatment of severe fungal infections despite its undesirable side effects. The toxicity of the drug has been related to its low solubility, more specifically to a self associated form termed molecular aggregate. According to the general conviction, the biological action of the drug is based on the formation of membrane pores that considerably affect physiological ion transport, especially of potassium ions. This work reports a study of the effect of potassium (K^+) and sodium (Na^+) ions on the self-aggregation of AmB in the lipid membrane environ-

ment. Mixed Langmuir monolayers of AmB and dipalmitoylphosphatidylcholine (DPPC) were investigated by recording surface pressure-area isotherms spread on aqueous buffers containing physiological concentration of K^+ and Na^+ ions. The analyses of π–A and compressional modulus curves indicate the existence of interactions in the AmB-DPPC system. The strength of the AmB-DPPC interactions and the stability of the mixed monolayers were examined on the basis of the excess free energy of mixing values. The results obtained proved a high affinity of AmB towards lipids in the presence of K^+ than Na^+ ions. The most stable monolayers were produced from molecules of AmB and lipid with the 1:1 and 2:1 stoichiometry in the presence of K^+ and Na^+ ions, respectively.

The understanding of these processes at the molecular levels and the possibility of modulating the aggregation state of AmB should contribute to elucidatation of the mechanisms of action and toxicity of this widely used antibiotic. The results of the research presented in this work are potentially valuable in respect to develop more efficient and less toxic preparations of AmB.

4. Verification of an atomistic computer model of the thylakoid membrane

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Thylakoid membranes of higher plant chloroplasts are the most abundant membranes found in Nature. Their main lipid component, constituting more than 75% of all lipids, are galactolipids. The most common type of galactolipids found in the thylakoid membrane is 1,2-di-*O*-acyl-3-*O*-β-D-galactopyranosyl-*sn*-glycerol (MGDG), whose head group consists of a single molecule of β-D-galactose. Vast majority of the thylakoid galactolipids have γ-linolenic chain both in the sn-1 and sn-2 position.

An atomistic computer model of the thylakoid membrane used in this study is a bilayer consisting of 128 MGDG molecules generated in over 200-ns molecular dynamics (MD) simulation using Gromacs 4.0 package and OPLS-AA force field. Because of the lack of a detailed structural and dynamical experimental characterization of MGDG bilayers, validation of the simulated system was problematic. To verify the model, we carried out a comparative study, where the reference system was a well studied by a variety of experimental methods bilayer consisting of 128 dioleoylphosphatidylcholine (DOPC) molecules and MD-simulated also for over 200 ns using the same software and the same force field as the MGDG bilayer. Both bilayers were simulated in identical conditions (temperature of 310K or 295K and pressure of 1 bar). We presume that positive verification of the computer model of the DOPC bilayer will imply positive verification of the MGDG bilayer. The main focus of the presented study is derivation of the structural parameters characterizing both bilayers; among them are: the membrane thickness, average area per lipid, order parameter profile along hydrocarbon chains, and tilt of hydrocarbon chains.

5. Melatonin applied into the cucumber seeds modifies antioxidant enzymes activity during germination

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Seeds are the plant material difficult to investigate because their population is usually heterogenic and they germinate nonuniformly. Seeds conditioning is one of the techniques solving this problem. It equalizes the rate of seeds germination and kinetics of plant emergence. Plants after conditioning exhibit better vigor even under stress conditions. Seed priming may involve supplementation with growth regulators (hormones), protective substances (antibiotics, fungicides) and biostimulators (e.g. melatonin).

Melatonin (MEL) applied into cucumber seeds (*Cucumis sativus* L.) modifies seeds metabolism during germination. They exhibit much higher tolerance to secondary oxidative stress caused by chilling. Biological effects of oxidative stress are *inter alia*: lipid peroxidation and oxidative damage of proteins and DNA. If plants have an efficient enzymatic antioxidant system, reactive oxygen species can be neutralized. It has been shown that MEL can increase activity of antioxidant enzymes, such as: superoxide dismutase (SOD) and glutathione reductase (GSSG-R). This positive effect was confirmed by biochemical test of hydrogen peroxide (H_2O_2) assay and tests of histochemical visualization: staining for hydrogen peroxide (H_2O_2) and superoxide radical (O_2^-) in cucumber axes tissues.

There is a still lack of information explaining clearly the role of MEL in plant physiology. However recent results indicate its role as a plant biostimulator of antioxidant enzyme activities.

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6. Effect of safeners on erythrocyte hemolysis induced by herbicides

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Alachlor and acetochlor are chloroacetanilide herbicides mainly used for preemergence control of annual grasses and small seeded broadleaf weeds in corn and soybean. U.S. Environmental Protection Agency (USEPA) classified alachlor as a probable human

carcinogen. It can induce lung tumors in mice and stomach, thyroid and nasal turbinate tumors in rats. Acetochlor has also been reported as a potential human carcinogen.

Pesticides do not act selectively and most of them are also toxic to crop plants, animals and humans. Safeners could be a helpful solution, since they selectively protect crop plants from herbicide damage and did not reduce their activity in target weed species. There is no information about probable human toxicity of safeners.

The aim of this study was to examine the possible protective effects of safeners against toxicity of chloroacetanilide herbicides in human erythrocytes. Hemolysis of erythrocytes was measured as an important index of their membrane damage, causing a release of hemoglobin and other internal components into the surrounding.

Erythrocytes were incubated for 24 hours at 37°C with different concentrations of herbicides (alachlor or acetochlor), their intermediate metabolites (MEA or DEA) and safeners (dichlormid or mefenpyr). The safeners were used alone or in combination with each of the herbicides at 1:1 ratio.

Our results showed that herbicides at the dose of 1000 μM, after 24 hours of incubation, caused hemolysis of human erythrocytes in contrast to their intermediate metabolites, which did not change erythrocyte integrity. Safeners, even at millimolar concentrations, were not toxic for human erythrocytes, but they did not display any protective effect on erythrocyte damage caused by herbicides.

7. Oxidative stress induced in erythrocytes by herbicides – effect of safeners

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The general population is inevitably exposed to the residues of pesticides and safeners, and to their physical and biological degradation products in air, water and food. Safeners are used commercially to improve herbicide selectivity between crop and weed species. They could be the most effective because of their structural similarity to herbicides. Although there is no conclusive mechanism explaining action of the available safeners, it seems that they improve crop tolerance to herbicides by regulating the expression of genes involved in metabolism of these compounds. Probably safeners might also act by competing with herbicides for the same site of action.

In our study we investigated the effect of safeners on some of the parameters of oxidative stress generated during exposure of human erythrocytes to herbicides. Peroxidation of lipids of erythrocyte plasma membrane was measured on the basis of the amount of TBARS and conjugated dienes. Additionally, catalase activity was estimated.

Erythrocytes were incubated with different concentrations of herbicides (alachlor/acetochlor) and safeners (dichlormid or mefenpyr) for 24 hours at 37°C. When the combination of a safener and a herbicide was used, both compounds (1000 μM each) were mixed at a 1:1 ratio.

We have found that herbicides caused an increase in the amount of conjugated dienes at a high (1000 μM) concentration only, but barely affected activity of catalase. Safeners did not induce any changes in both lipid peroxidation and catalase activity. They, however, reduced lipid peroxidation, induced by herbicides, to the level of control, which suggests the antioxidative protection by these compounds. Such an effect was not observed for catalase activity.

8. Toxicity of tin organic compounds towards erythrocyte membrane

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The aim of the studies was to determine the relationship between toxicity and location of the tin organic compounds: triphenyltin chloride (TPhT), tributyltin chloride (TBT), diphenyltin dichloride (DPhT) and dibutyltin dichloride (DBT). To this end were investigated erythrocyte shapes induced by the tin compounds, using the electron and optical microscope, and the fluorimetric method with the three probes: Laurdan, TMA-DPH and DPH.

Earlier studies performed with the ATR ETR method for this group of compounds have shown that TBT is the most active compound in its interaction with the erythrocyte membrane. It penetrates the membrane causing the greatest dehydration of the membrane lipids at the level of carbonyl and phosphate groups. From our microscopic observations it follows that the organic tin compounds induce the formation of echinocytes mostly, which testifies that the compounds accumulate in the outer monolayer of the membrane lipids. The fluorimetric studies have shown that the compounds studied are present in the hydrophilic part of the outer monolayer and at the boarder between hydrophilic and hydrophobic regions of the erythrocyte membrane. Only TBT, incorporating deeper into the monolayer, i.e. in its hydrophobic region, alters the packing order of the alkyl chains. Shape changes result in changes in packing of the polar heads of lipids in the outer lipid layer of membrane. The high toxicity of TBT, documented earlier, is thus the result of the compound's incorporation in the hydrophobic region.

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9. Effect of extracts from *Uncaria tomentosa* on survival, morphological changes and the process of apoptosis in human lymphocytes

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Uncaria tomentosa is a climbing plant, which belong to the madder family (*Rubiaceae*). Majority of available studies concerned the effect of *Uncaria tomentosa* extracts on cancer cells (*in vitro*). Therefore, based on those studies it is difficult to foresee any effect of the tested preparations on regular cells or function of whole organism.

The purpose of this study was to determine the effect of *U. tomentosa* extracts on structure and function of human peripheral blood lymphocytes obtained from healthy donors. Lymphocytes are the most important cells of the immunological system responsible for protection of the organism against pathogens, and participate in maintenance of the homeostasis. In this work, the effect of ethanol- and water-based extract from *U. tomentosa* leaves and bark was tested. Incubation of the lymphocytes with tested extracts from leaves and bark of *U. tomentosa* decreased human lymphocyte survival, decreased lymphocyte size and increased cells granularity. The tested extracts caused apoptotic changes in human lymphocytes, as well as increased count of the cells with reduced mitochondrial transmembrane potential. The effect of the tested extracts depended on their type and concentration. Ethanol-based extracts from the leaves and the bark showed higher toxicity for the lymphocytes. The results of our study suggest that the effect of the preparation (extract) introduced to an organism does not necessarily have to be associated with its direct toxicity for cancer cells. The obtained results also shown cytotoxic effect of the extracts on the incubated cells, but the observed cytotoxicity depended on dose of the tested extracts. Evaluation of pharmacologically active doses that should be used for therapeutic reasons is very difficult. Our results demonstrate that cytotoxicity of tested extracts occurred only at very high concentrations, which potentially cannot be present in blood (*in vivo)*. Those results, combined with the information concerning the effect of extracts from *U. tomentosa* on red blood cells (see next abstract), reveal that the effect of the extracts is closely related to a type of the cell. It was shown that nucleated cells are much more susceptible to effect of these compounds, in which cytotoxic effect was observed.

10. Hemolysis induction and hemoglobin oxidation in red blood cells incubated with extracts from *Uncaria tomentosa*

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The progress observed in the last few decades in phytochemical and phytotherapeutic studies has been largely driven in the direction of development of effective therapeutic methods, which are useful in treating of numerous diseases, especially cancer. Development of many diseases results from pollution of the environment with many xenobiotics including pesticides. These substances reveal noxious effect on human health. Their activity includes hepatotoxocity, changes in development of the circulatory and respiratory system, and development of various carcinomas. *Uncaria tomentosa* (Willd.) DC is a lignified climbing plant from South and Central America, which (under the name of "vilcacora" or "cat's claw") has become highly popular in Poland and in many other countries due to its proven immunostimmulatory and antiinflammatory activity and also in respect to its anticancer and antioxidative effects. The purpose of this study was to evaluate the inhibitory effect of the extracts from *U. tomentosa* on hemolysis induction and hemoglobin oxidation provoked by selected xenobiotics, i.e. 2,4-dichlorophenol (2,4-DCP) and catechol, to demonstrate their previously postulated antioxidative properties, and to show that extraction with various solvents has a significant modulatory effect on pharmacological activity of the extracts studied. To execute our experiment, ethanolic and water extracts from *U. tomentosa* leaves and bark were used. The results of the study confirmed antioxidative effect of *U. tomentosa*. The extracts caused reduction of hemolysis and hemoglobin oxidation provoked by 2,4-DCP and catechol. A difference in the effect of the extracts studied was observed. Ethanol-based extracts revealed more pronounced ability to inhibit hemolysis and hemoglobin oxidation. A conclusion is drawn that water-based extraction does not allow to exploit the total antioxidative potential of the plant studied.

11. Interaction of xanthohumol and isoxanthohumol with phosphatidylcholine bilayers

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Xanthohumol and isoxanthohumol are prenylated chalcones isolated from hops. Hops are used to add

bitterness and flavor to beer. Xanthohumol was characterized as an anticancer agent. Prenylflavonoids from hops might also be used in prevention of menopausal "hot flashes" and osteoporosis. In the present study differential scanning calorimetry and fluorescence spectroscopy have been used to investigate interaction of xanthohumol and isoxanthohumol with a model membrane. The influence of xanthohumol on thermotropic properties of DMPC model systems was significant, even at low compound:lipid molar ratios, as it reduced temperature and cooperativity of the main phospholipid phase transition. Isoxanthohumol also interacted strongly with DMPC bilayers, moreover the appearance of two sub-peaks at higher modulator:lipid molar ratios suggested phase separation. Additionally, several fluorescent probes localized in different membrane segments were used to investigate influence of these flavonoids on PC bilayers. We used DPH, which fluorophore locates deep within the bilayer, Laurdan which locates near glycerol backbone and Prodan which is positioned closer to the bilayer surface than Laurdan. Strong quenching of all fluorescent probes by xanthohumol was observed. The effect of the studied compounds on PC bilayers was studied both as a function of drug concentration and temperature. Both compounds influenced fluorescence intensity of Prodan to a greater extent than that of Laurdan. Additional studies on Laurdan generalized polarization dependence on the excitation wavelength revealed isoxanthohumol-induced phase separation in PC membranes. Molecular modeling was also performed in order to identify physicochemical parameters of the studied compounds important for their interaction with phosphatidylcholine bilayers.

12. Antioxidant potency of extracts from leaves of fruit trees and bushes

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For many years now, throughout the world studies are conducted aimed at discovery of compounds which are able to control the level of free radicals without exerting any side effects. The human body is incessantly exposed to the action of free radicals, which are blamed for causing many civilization-associated diseases. For this reason it is extremely important to supply the organism with substances that reduce the level of free radicals.

We conducted spectrophotometric and fluorimetric studies on the antioxidant activity of polyphenolic extracts from leaves of strawberry, blackberry and apple, using erythrocyte ghosts as the oxidation target. The antioxidant potency was determined as the ability to inhibit membrane lipids peroxidation and scavenge free radicals induced by UVC and AAPH radiation in the presence of the compounds studied. In the spectrophotometric method, lipid peroxidation was assayed on the basis of MDA concentration released in the peroxidation process, while in the fluorimetric method it was done on the basis of DPH-PA probe fluorescence intensity.

The studies have shown that the compounds markedly reduce the level of free radicals in erythrocyte ghosts suspensions and inhibit membrane lipid oxidation.

It was documented that the extracts studied possess very good properties as free radical scavengers, and could well be applied as dietary supplements and additives to cosmetics.

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13. Effect of quercetin on oxidative stress induced in MCF-7 breast cancer cells by doxorubicin and taxanes

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Combination of anthracyclines with taxanes, e.g. doxorubicin (DOX) and docetaxel (DTX), is one of the most common chemotherapy choice for many solid tumors. The main side effect of such chemotherapy is cardiotoxicity, which depends mainly on oxidative stress generated by these drugs. Flavonoids are the most common known antioxidants that may contribute to protection of cells against oxidative stress.

The objective of this study was to assess the effect of quercetin on oxidative stress generated by DOX and DTX in MCF-7 breast cancer cells. The amount of carbonyl groups as a marker of protein damage and the amount of hydroperoxides as a marker of lipid peroxidation were estimated. Cells suspended in HBSS buffer were incubated with the investigated compounds for 3 h (Q) or 2 h (drugs). One-hour preincubation with the flavonoid followed by 2-h incubation with the drugs was also applied.

In control cells, low concentrations of Q caused an increase in the amount of carbonyl groups while its high concentrations decrease the level of protein carbonyls. The flavonoid reduced the level of hydroperoxide in a concentration-dependent manner, i.e. the effect decreased with an increase of Q concentration. DOX increased 7-fold the level of carbonyl groups, while the effect of DTX was considerably lower. Of both drugs only DOX caused elevation of hydroperoxides (1.5-fold). Combination of DOX with DTX caused a 3.5-fold increase in the level of carbonyl groups, but only slightly changed amount of hyperoxides. Quercetin applied together with the drugs reduced both parameters of oxidative stress. The protective effect of quercetin was dependent on the flavonoid concentration. Greater protection was achieved with higher concentrations of the flavonoid.

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14. The *in vivo* effect of nitroxide Pirolin and anticancer drugs doxorubicin and docetaxel on activity of antioxidant enzymes in rat heart

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Anticancer treatment is based on the induction of apoptosis, in which ROS overproduction is often involved. An anthracycline, doxorubicin (DOX) and a taxane, docetaxel (DTX), used as single agents or in combination, display strong side effects, e.g. cardiotoxicity, developed mainly by induction of oxidative stress in cardiomyocytes. Cardiotoxicity reduction might be achieved by implementing chemotherapy with antioxidant(s), e.g. nitroxides.

We evaluated effects of a nitroxide Pirolin (PL) on the oxidative stress induced in rat hearts *in vivo* by DOX, DTX or their combination. Wistar rats were injected *i.p.* with the tested compounds and four days later the hearts were removed. Activities of catalase (CAT), total superoxide dismutase (SOD), MnSOD and CuZnSOD in heart homogenates were assayed.

DOX significantly increased activities of all enzymes, except CuZnSOD, while DTX effect was negligible. Combination of both drugs boosted activities of CAT, SOD and MnSOD, and to a lesser degree activity of CuZnSOD. Pirolin alone increased MnSOD activity only. PL profoundly enhanced changes in CAT, SOD and CuZnSOD activities induced by DOX. Combination of PL with DTX did not cause any noteworthy changes. A notable decrease in enzyme activity was observed for combination of PL with both drugs (vs. DOX + DTX).

We conclude that PL can display various effects *in vivo* depending on the combinations used. It strongly increased activities of antioxidant enzymes in combination with DOX but significantly decreased enzyme activities in combination with DOX and DTX.

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15. Spectroscopic studies of antioxidative properties of cosmetics

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Free radicals play important role in both health and disease [1, 2]. Reactive oxygen species (ROS) are implicated in many disease and photoageing. In skin free radicals cause collagen degradation and epidermis damages [1, 2]. There is a lot of evidence about antioxidants role in disease prevention [3]. It is supposed that application of antioxidants in cosmetic products can delay the ageing processes and also reduce the risk of precancerous changes [4].

The aim of work was to evaluate of antioxidative properties of cosmetics. The EPR spectroscopy was used to define antioxidative potential of tested samples. DPPH signal intensities after cosmetics samples adding were compared. Free radicals were studied by electron paramagnetic resonance spectroscopy at X-band (9.3 GHz) with magnetic modulation of 100 kHz. EPR spectra were measured by Radiopan (Poznań) spectrometer with Rapid Scan Unit (Jagmar Firm, Kraków). The measurements were done with microwave power in the range 2.2–70 mW at room temperature. Lineshape of the EPR spectra and their parameters were analyzed. Integral intensity, amplitude and linewidth of the spectra were shown.

The EPR spectra of DPPH with studied cosmetics differ in shape and parameters. Combining few antioxidants substances in cosmetics increases free radicals scavenging.

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16. Influence of some aliphatic tin compounds on the electrical properties of model membranes

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The aim of this study was to investigate the effect of selected organic tin chlorides $(C_4H_9)_3SnCl$ (TriBT), $C_4H_9SnCl_3$ (BT), $(CH_3)_3SnCl$ (TriMT) and CH_3SnCl_3 (MT) on the transmembrane voltage of the model membranes in the form of negatively charged filters produced by the Synpor company, impregnated with lauric acid butyl ester. Filter diameter was 1.5 μm and the thickness 50 μm. Membrane separates the reference chamber from the measuring chamber. In the initial moment the chambers were filled with KCl solution at a concentration of 0.01 mM/dm^3. Membrane voltage induced by the studied tin compounds introduced into the measuring chamber was measured using Keithley 6517 electrometer and Ag/AgCl electrode system.

The selected test compounds generate membrane voltage, which increases in the first phase up to same maximum value and then (except for MT compound) decreases and tends to stabilize. In the case of TriMT double voltage peaks were observed, extended in time, for the smallest concentrations used in studies of this compound. At the largest concentrations of TriBT, membrane voltage value decreases steadily after reaching its maximum value and changes the polarity.

It can be expected that maximum voltages result from penetration of tin organic cations into the interior lining, followed by chlorine anions left in the measuring chamber. The change in membrane polarity may be caused by the movement of chloride ions across the membrane, from the measuring chamber to the reference chamber.

The obtained results permit to conclude that both butyltin compounds generate greater transmembrane voltage than the metyltin compounds. The highest concentrations TriBT used in the study, produce a certain type of ion channels in the membranes, allowing for the transport of chlorine through a membrane. The results obtained for the tested metyltin compounds indicate that the interaction of these compounds with the membrane occurs at the surface. The effectiveness of interaction of the tested compounds with model membranes increases with increasing length of hydrocarbon chains in their molecules.

17. DNA damage and repair in cancer cells – comparison of anthracyclines of Ist and IInd generations

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Doxorubicin (DOX) and aclarubicin (ACL) anthracycline antibiotics are the most widely used antineoplastic agent for the treatment of solid tumors (DOX) and leukemias (ACL). Their cytotoxic effects are commonly accepted to be mainly due to DNA-intercalation. In order to get an insight into the mechanisms of cell death induced by these drugs in human hepatoma and lung cancer cells, we compared cyto- and genotoxicity of ACL and DOX in HepG2 and A549 cancer cell lines. Genotoxicity of anthracyclines, usually expressed as they ability to induce DNA damage, is often essential for mutagenesis and malignant transformation. Thus, we studied the extent of DNA damage and repair by the single cell gel electrophoresis (comet assay). In some experiments we preincubated the cells with verapamil (well known inhibitor of Pgp) before ACL or DOX treatment. Amount (%) of DNA in the comet tail was considered as a marker of DNA damage. Cytotoxicity of ACL and DOX was studied with neutral red (NR) and MTT assays, after incubation of cells with a range of drug concentrations for 1, 3 and 12 h.

We have found that both cell lines were significantly more sensitive to ACL than to DOX. The sensitivity of the cells to ACL (A549 > HepG2) followed an inverse order than their sensitivity to DOX (HepG2 >A549). In both types of cancer cells ACL caused more DNA damage than DOX, but generally hepatoma HepG2 cells were more resistant to anthracycline treatment than non-small lung cancer A549 cells. The level of DNA damage increased with drug concentrations and the length of the treatment time. Verapamil markedly enhanced DNA damage induced by DOX, but not by ACL. 30 min-incubation of cells in fresh medium following drug treatment reduced the percentage of damaged DNA, which suggests effective repair processes in the investigated cancer cells.

18. Is it possible to store platelets at low temperature?

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Platelet concentrates are routinely stored for five days at 22°C under horizontal agitation. Unfortunately these storage conditions are associated with increasing sepsis from bacterially contaminated concentrates that are reduced at low temperatures. On the other hand, the exposure of the platelets to cold causes depolymerisation of microtubules and loss of the discoid shape. The recovery and survival of the platelets stored at 4°C is decreased relative to platelets stored at room temperature. The experiment in blood bank in Wroclaw was conducted to examine, if concentrates stored for eight days at room and low temperatures modified by exposure (every 6 hours) to near infrared (NIR) radiation have favorable properties compared to control samples (non-irradiated). We analyzed samples by measurements of extracellular pH, number of platelets, platelet aggregation and using flow cytometry. Preliminary results obtained using a platelet aggregometer did not show significant changes between samples from concentrates stored in different conditions, while pH measurements and flow cytometric results revealed differences between samples originated from concentrates at low and room temperatures. The measurements of number of platelets during experiment and after incubation at 37°C showed evidently the influence of NIR radiation on platelet concentrations.

Our preliminary findings indicate that NIR radiation can favorably influence cells increasing their survival and viability after warming. These effects were seen in concentrates stored both at a low and room temperature, however stronger in the latter case. Precise analysis needs further studies.

19. The influence of the hydroxyproline content on the denaturation temperature of collagen

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The possibility of replacing bovine collagen with fish collagen is connected with studies on thermal stability of fish skin collagen in the solid state. It requires studies on free, bound water and structural water release processes as well as studies on phase transitions in such processes as vitrification and denaturation.

Protein thermal stability, which can be described by denaturation and vitrification temperatures is related, among others, to the primary structure of collagen. The amino acid sequence and the content of characteristic amino acids affect the thermal stability of the protein structure. In the case of collagen, hydroxyproline is such an amino acid. Its higher content in collagen causes a rise in denaturation temperature. The presence of hydroxyproline, as a characteristic amino acid of collagen, is used for quantitative evaluation of the collagen content.

The fish skin (FS) collagen used in the study was obtained by means of acidic hydration methods. As a control material bovine Achilles tendon (BAT) collagen was used (Sigma Aldrich). The amount of hydroxyproline in FS collagen and BAT collagen was determined by the Stegmann spectrophotometric method, modified by Hurych and Chvapil. Denaturation temperature was determined using temperature dependence of electric conductivity. Samples were cyclically heated in order to eliminate peaks appearing during preceding heating runs. This allows to conclude if the occurring processes were reversible or irreversible. Every successive heating run was finished above the temperature of the peak recorded in the preceding heating run. In the first heating run samples have been heated in the range of 290–380 K, where free and bound water was released. In the second cycle, samples were heated up to 510 K and the process of thermal denaturation was observed. The heating run above 510 K caused thermal decomposition of the material.

Table 1. Dependence of denaturation temperature on hydroxyproline content

Collagen	FS	BAT
Denaturation temperature [K]	443	487
Hydroxyproline content [g/100 g protein]	5.68	8.15

Determined denaturation temperatures and hydroxyproline contents for FS and BAT collagens are shown in Table 1. Applying the Welch test, it was found that differences in the hydroxyproline content were statistically significant ($p = 0.0063$). The obtained results prove the hypothesis that the higher the hydroxyproline content, the higher the denaturation temperature.

20. The extract from parasitic fungi used in the unconventional medicine affect the red blood cells by disruption of the membrane structure

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Two parasitic fungi *Piptoporus betulinus* and *Inonotus obliquus* are used in unconventional medicine in whole Europe. They are used by oral administration of hot-water or ethanol extracts from whole fructification. Their antitumor activity was inferred from studies of their effects on HeLa, HT-29 and H549 cells and of animal models of leukemia. An antiinflammatory effect of the extracts was also reported. Biologically active compounds were extracted from both species and partly identified. In spite of all reports of beneficial actions and high antioxidant activity of the extracts there are no reports on their influence on the condition of a healthy organism and its structures. In this study we investigated the potential damage of the erythrocyte membrane caused by water extract obtained from commercial preparation of both fungi. Our observations prove that components of these extracts can incorporate into erythrocyte membrane and powerfully affect it causing hemolysis and membrane protein damage.

21. The photoacoustic profilometer

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One of the advantages of photoacoustic spectroscopy (PAS) is the possibility to analyze a given sample layer-by-layer without affecting its physically structure. In the PAS measurements the sampling depth L decreases with the modulation frequency increase. The sampling depth is given by:

$$L = \sqrt{\frac{D}{\pi \times f}}$$

where: D is the sample's thermal diffusivity, and f the light modulation frequency. In the studies presented, a new experimental setup based on a lock-in EG&G 5015 amplifier and functional generator FG-503 was used. For controlling the apparatus as well as for acquisition of the measured data the LabView environment was used. A part of the software responsible for controlling the generator was originally created, whereas the driver for the phase-sensitive amplifier, available at the National Instruments website, was

modified. Both, the function generator and phase-sensitive amplifier are connected to the PC-class computer by RS-232 serial ports. The all-in-one programming approach provides a comfortable and efficient work environment that ensures an effective collection of the photoacoustic signals of analyzed samples.

The apparatus allows for comfortable measurements of a change in concentration of the given substance in a sample as well as its of thermal parameters versus changing depth. It also enables measurements of, e.g., the change of efficiency in photo-chemical processes depending on the distance from the sample surface. For materials with thermal parameters similar to those of water, i.e. almost all biological samples, the useful range of depth reaches several dozen micrometers below the sample surface. In the presented studies, we provide a few examples of experimental results of a change in the thermal parameters of an oil-water mixture and with variability of efficiency of energy trapping in the photosynthesis process as a function of profiling depth.

22. Analysis of voltage pulsations caused by hydrodynamic instabilities in near membrane area

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Results of measurement of voltage between two electrodes dipped directly into ionic solutions in membrane systems show appearance of hydrodynamic instabilities in the membrane system, manifesting itself by pulsations of that voltage. Pulsations of voltage were observed in the cases of membranes placed in horizontal planes and with solutions with higher density over the membrane. The main characteristics of pulsations (frequency, amplitude) depend on initial conditions in the membrane system (the moment of turning off mechanical stirring of solutions), such as initial ratio of solute concentrations on the membrane. Fourier analysis of voltage signals do not show unequivocally frequencies prevailing in the analyzed signals because of random processes of hydrodynamic instability appearance in the layers of solution with density gradient directed oppositely to the vector of gravitational field. Taking this problem into consideration the procedures such as analysis of quantity and amplitude of pulsations in definite time interval, graphic presentations of delayed signal in time as a function of that signal (delayed graphs) and phase graphs of voltage signals were elaborated. This analysis shows that increase of density difference between chambers at the initial moment in membrane system causes increase of frequency and amplitude of pulsations. Besides phase graphs (derivative of signal as a function of signal) as well as delayed graphs in determined intervals of time, changes of pulsations character in time after hydrodynamic instability appearance in membrane system can be seen. The evolution of concentration boundary layers in time, which thickness and density gradient in that layer depend on substance diffusion and on conditions of hydrodynamic instability appearance, is the cause of such voltage characteristics.

23. Time and pressure characteristics of membrane potentials in non-homogeneous conditions for electrolyte solutions

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The concentration polarization of microbial cellulose membrane perpendicular to gravitational field, in the conditions of settled pressure difference (ΔP) on the membrane was studied. The method of voltage between Ag|AgCl electrodes, dipped directly into KCl solutions on both sides of the membrane was used. The substantial influence of the pressure difference on the membrane on time characteristics of voltage for both configurations of the membrane system: with lower density over (A) and under (B) the membrane was found. The ratio of KCl concentrations at electrodes surfaces at states settled in a definite moment of time after turning off mechanical stirring of solutions (C_i^*/C_e^*) was counted from voltages in the membrane system. C_i^*/C_e^* is the nonlinear function of ratio of KCl concentrations in the membrane system at initial moment (C_h/C_l) and depends on the pressure difference on the membrane and configuration of the membrane system. The shape of dependence $C_i^*/C_e^* = f(\Delta P)$ was asymmetric and for pressure gradient directed in opposite direction to the gradient of KCl concentration on the membrane, a maximum of $C_i^*/C_e^* = f(\Delta P)$ was observed. Increase of gradient of KCl concentration on the membrane at initial moment caused a shift of maximum to higher values of pressure difference on the membrane. Besides, the dependence of the interval of time needed for appearance of hydrodynamic instabilities in the membrane system after turning off mechanical stirring (T_P), as a function of settled pressure difference on the membrane for B configuration was measured. The hydrodynamic instabilities in the membrane system manifested as voltage pulsations, resulted from disturbance of ions concentrations at electrodes surfaces. It was found that the direction and value of pressure gradients on the membrane causes substantial change of T_P in comparison with T_P in the case without a pressure gradient on the membrane. The dependences $C_i^*/C_e^* = f(\Delta P)$ and $T_P^{-1} = f(\Delta P)$ can be connected with disturbance of time evolution of concentration boundary layers by settled pressure gradients on the membrane.

24. Reversible deactivation of platelets induced by near infra red radiation

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Blood is responsible for transport, defense against infections and correct functioning of entire organism. Acute internal organ failures require use of their artificial equivalents for example ECMO, hemodialyzer or Cardiopulmonary bypass. They take out the blood outside of the organism in order to purify and oxygenate it. Morphotic blood elements abiding in extracorporeal circulation are vulnerable to damage.

The aim of this *in vitro* research was to determine the influence of near infra red radiation (NIR) on platelets in whole blood at 37°C.

We investigated platelet count and activation with respect to various dose and times of exposition to NIR. Number of platelets was determined by a direct method using an optical microscope and Burker chamber. The extent of platelet activation was measured by agonist induced (ADP, collagen) aggregation with a Chrono-Log whole blood impedance aggregometer. Both porcine and bovine whole blood was used.

Near infra red radiation reduces loss in the platelet count. Variation of parameters of radiation and time of exposure changes only the intensity of influence and not its character. Alteration of capability to aggregate of the platelets is not persistent. Repetition of exposure to NIR results in maintaining decreased loss of platelet number during the experiment, however their ability to activate is not blocked permanently.

25. Lysenin interaction with sphingomyelin-rich membranes – monomolecular layer technique and surface plasmon resonance studies

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The mode of action of a large group of biologically active proteins named pore-forming toxins (PFTs) is based on their unique ability to exist both in a water-soluble state or as an integral membrane pore, that is permeable to various components. Cytolysis induces by PFTs is a result of a multistep mechanistic process including secretion of toxin molecules from the host, their binding to the membrane, oligomerization by protein-protein interaction and pore formation with insertion into the cell membrane. One of the novel member of this class of toxins is lysenin produced by the earthworm *Eisenia foetida* to protect against killing invaders. The lysenin polypeptide chain is 297 amino acid long with the calculated molecular weight of 41 kDa. In case of lysenin, protein attachment to the membrane surface is a very specific process. The strong lysenin affinity toward sphingomyelin, a major plasma membrane lipid in an animal cell, attributes sphingomyelin the role of a lysenin receptor in membranes. In order to analyze the details of lysenin sphingomyelin-rich membrane interaction, the series of measurements with wild-type and mutant lysenin forms have been carried out. Comparison of the results of adsorption of the wild-type and non-lytic form of lysenin at the air/water interface shows differences in the molecular structure of both proteins what causes changes in the oligomerization process which are the reason of forming different structures in lipid membrane. The results of surface plasmon resonance study show that the mutation has also an effect on protein selectivity and binding process.

26. Changes of liposomal membrane fluidity induced by amyloid peptides. A spin label study

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Ordered protein aggregation with amyloid fibril formation and its accumulation in body tissues particular in brain lead to the range of the severe neurodegenerative diseases like Alzheimer's (AD), Parkinson's and others. There are hypotheses that non-specific interaction between Aβ proteins and neuronal membranes plays an important role in the conformational changes and aggregation of these peptides. In this work we analyzed the effects of amyloid beta peptides on fluidity of liposomal membranes using electron spin resonance (ESR) technique. For 5-doxylstearic acid (5-DSA) and 16-doxylstearic acid (16-DSA) spectra, the amplitudes of the h_{+1} and h_0 were measured whose ratio is related to the fluidity of the local environment of the labels. PC/Chol/PE/PS/SM, 55:25:10:5:5, (Mol/Mol) liposomes prepared by extrusion method were preincubated with Aβ at peptide:lipid molar ratio 1:100 or 1:10 for 30 min at t = 45°C (above the PC main transition temperature). The spin labels were added to the liposomes in the ratio of 1:100 (Mol/Mol) and incubated for 15 min at room temperature.

The ratio h_{+1}/h_0 was reduced upon interaction of LUVs with amyloid peptides indicating a decrease of mobility for both spin labels. For 5-DSA and 16-DSA no significant changes caused by normal or aggregated form of $A\beta_{1–28}$ were observed. Only non aggregated form of $A\beta_{25–40}$ induced a significant decrease in the

h_{+1}/h_0 ratio of 5-DS. This effect was more pronounced for peptide:lipid molar ratio 1:100. It has also been shown for a 5-DSA labeled membranes containing $A\beta_{1-40}$ that membrane fluidity preferentially decreased for aggregated form of amyloid. The membranes containing 16-DS did not show statistically significant difference between control and $A\beta_{1-40}$ treated samples. But in the case of aggregated form of $A\beta_{1-40}$ (P:L ratio: 1:10) a decrease of membranes fluidity existed that suggests that aggregated form of AB were more active in comparison with non aggregated peptide fragments in both external and internal regions of liposomal membrane.

Obtained results show that the investigated peptides affect the bilayer order of liposomal membrane. The incorporation of Aβ fragments into the model lipid vesicles decreases the bilayer fluidity.

27. Poli(amido)amine dendrimer PAMAM G2.5 as a potential modulator of the intracellular calcium concentration

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Mitochondria play a pivotal role in the intracellular Ca^{2+} signaling by taking up and releasing the ions upon specific conditions. The potential cytotoxicity of Ca^{2+} ions has been recognized for about three decades notwithstanding, the exact mechanisms of calcium cytotoxicity remain controversial. This cytotoxicity is believed to manifest itself via different mechanisms. In the present investigation, we attempted to study the protective activity of anionic PAMAM dendrimer G2.5 against deleterious effect of Ca^{2+} ions on isolated rat liver mitochondria. We hypothesized that low half-generation PAMAM dendrimers are less toxic than the higher and full-generation ones and hence they can be used as calcium ions chelators, thus protecting mitochondria against overload with Ca^{2+} ions.

Mitochondria isolated from Wistar rats were exposed to Ca^{2+} ions (at the concentration equal to its estimated IC_{50}) and to dendrimer G2.5 (at its neutral concentration) and several parameters of mitochondrial function were monitored with the use of the spectrofluorimetric technique. Briefly, the mobilization of calcium ions was detected using spectrofluorimetry with Calcium Green 5-N. Mitochondrial membrane fluidity and transmembrane potential were tracked using the staining with 1,6-diphenyl-1,3,5-hexatriene (DPH) and JC-1, respectively.

Obtained data revealed that PAMAM G2.5 used at the concentration of 5 μM is not able to effectively reduce the adverse effects of calcium ions on the mitochondria function. All the measured parameters that became reduced following the incubation with Ca^{2+}, were not improved in the presence of dendrimer G2.5. Therefore, based on these preliminary results, we conclude that calcium ions-induced mitochondrial damage and the subsequent liver dysfunction cannot be prevented by PAMAM G2.5.

28. Oxidative and nitrative modifications of plasma proteins isolated from breast cancer patients

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Breast cancer is the most popular and very heterogenous malignant cancer in women in Poland and in the whole world at both the clinical and molecular levels. The process of cancer formation and development is associated with loss of redox balance in the cell and overproduction of reactive oxygen and nitrogen species. In breast cancer patients, oxidative stress and oxidative changes in the cells take place. Plasma proteins play an important role in the pathomechanism of altered hemostasis in cancers, therefore, the aim of our present study was to evaluate oxidative/nitrative modifications of plasma protein by measurement of the level of different biomarkers of oxidative/nitrative stress such as carbonyl group, 3-nitrotyrosine, total antioxidant status in plasma from patients with invasive breast cancer, patients with benign breast diseases and healthy volunteers.

Plasma was isolated from healthy subjects and patients, hospitalized in Department of Surgical Oncology, Copernicus Memorial Hospital, Medical University of Łódź, Poland. Identification of the level of 3-nitrotyrosine and carbonyl groups in plasma proteins was performed by ELISA tests. The level of total antioxidant status in plasma was measured spectrophotometrically by of inhibition of oxidation of 2,2-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS).

We observed a statistically significant increase of 3-nitrotyrosine and carbonyl groups level in plasma proteins; a decrease of total antioxidant status in plasma from patients with breast cancer (compared to the healthy group) was also found.

The different modifications (nitration or carbonylation) of plasma proteins might be partly associated with modulation of structure and function of proteins involved in hemostasis of breast cancer patients.

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