XXI. WORKSHOP OF BIOPHYSICAL CHEMISTS AND ELECTROCHEMISTS

BOOK OF ABSTRACTS

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The sponsors of the Workshop of Biophysical Chemists and Electrochemists:

The organizers thank a lot to all this year's sponsors for the support, which enabled to organize this traditional conference: VisegradFund, Metrohm Czech Republic s.r.o., Institute of Biophysics of the Czech Academy of Sciences in Brno, Eppendorf Czech & Slovakia s.r.o., Chromservis s.r.o., CHROMSPEC spol. s r.o., MERCI, s.r.o., 2THETA ASE s.r.o., TRIGON PLUS s.r.o. and Czech Chemical Society, subdivision Brno.

An introductory word...

Dear friends, dear colleagues,

We cordially welcome you to our traditional meeting of biophysical chemists and electrochemists. This year's conference includes several peculiarities that I would like to mention. The 21st Workshop of Biophysical Chemists and Electrochemists:

- is not held on the University Campus in Bohunice, as has been the case in previous years, but in the Continental Hotel;
- is supported by VISEGRAD project (Science in the V4 countries - research of new sensors for the diagnosis of diabetes), in which Slovakia (UPJŠ Košice), the Czech Republic (MUNI Brno) and Hungary (University of Pécz) are involved;
- is thematically divided into 4 smaller sessions that are opened by invited lectures;
- takes place after a long pause caused by a covid pandemic and everyone is looking forward to a joint face-to-face dialogue.

The program is very rich; in addition to 4 plenary and 4 invited lectures, you will hear 49 contributions, of which 18 competition presentations in the "Young Scientists Session" and 21 presentations in the Poster Session section, to which all participants are invited before the gala dinner on Thursday evening. On Friday, the conference will be concluded with the announcement of three winners of the "Young Scientists' Session", one winner of the Emil Paleček Award, and one winner of the Poster Session.

We are glad that the covid traffic light did not complicate our event and that we have the opportunity to appreciate the power of dialogue without restrictions. We wish you a stimulating conference that will leave very nice memories in your minds and which will give you full of positive energy for your further scientific and pedagogical work.

In conclusion, I would like to thank the VISEGRAD fund, Slovak colleagues, who started this project led by prof. Renata Oriňáková, to all sponsors (Eppendorf, Trigon Plus, 2-Theta, Chromservis, Merci, the Czech Chemical Society), especially Metrohm, led by Ing. Peter Barath, the Institute of Biophysics with the director Professor Eva Bártová (Emil Paleček Award), and last but not least, to all those who will present and discuss their scientific knowledge. We could not hold a conference without you.

Welcome to Brno, welcome to Masaryk University, and enjoy this conference!

Please, don't forget to protect yourself from COVID!

Libuše Trnková

***Motto*:** *The most beautiful experience we can have is the mystery. It is the fundamental emotion that stands at the cradle of true art and true science*.

Albert Einstein

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XXI. WORKSHOP OF BIOPHYSICAL CHEMISTS AND ELECTROCHEMISTS

PLENARY LECTURES

QUANTIZED ELECTRODES

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Quantum electrochemistry is well-established area of interfacial science. It is used to interpret in quantum mechanical terms some macroscopic behavior of electrodes. In this lecture I will report on preparation[1] of electrodes in such a way that their certain properties are predictably quantized. These electrodes consist of polyaniline (PANI) as an isolation matrix in which individual atoms of gold and palladium are imbedded in such a way that their quantized energy states calculated *in vacuo* are preserved. As an illustration, quantized kinetics of oxidation of aliphatic alcohols by Pt/PANI*Au_2Pd_1 and Pt/PANI*Pd_2Au_1 system[2] will be presented. It will be shown that the order in which the the triatomic catalytic sites have been created plays a dominating role in their electrocatalytic behavior[3].

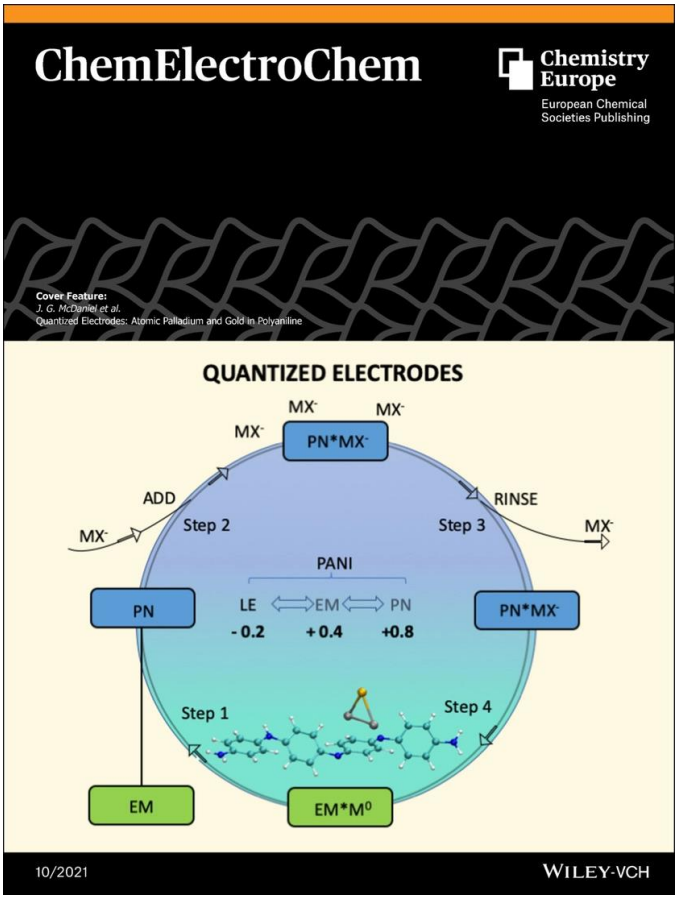

Figure 1: Quantized electrodes

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ELECTRONS IN LIQUID AMMONIA AND IN WATER: FROM BLUE ELECTROLYTES TO GOLDEN METALS

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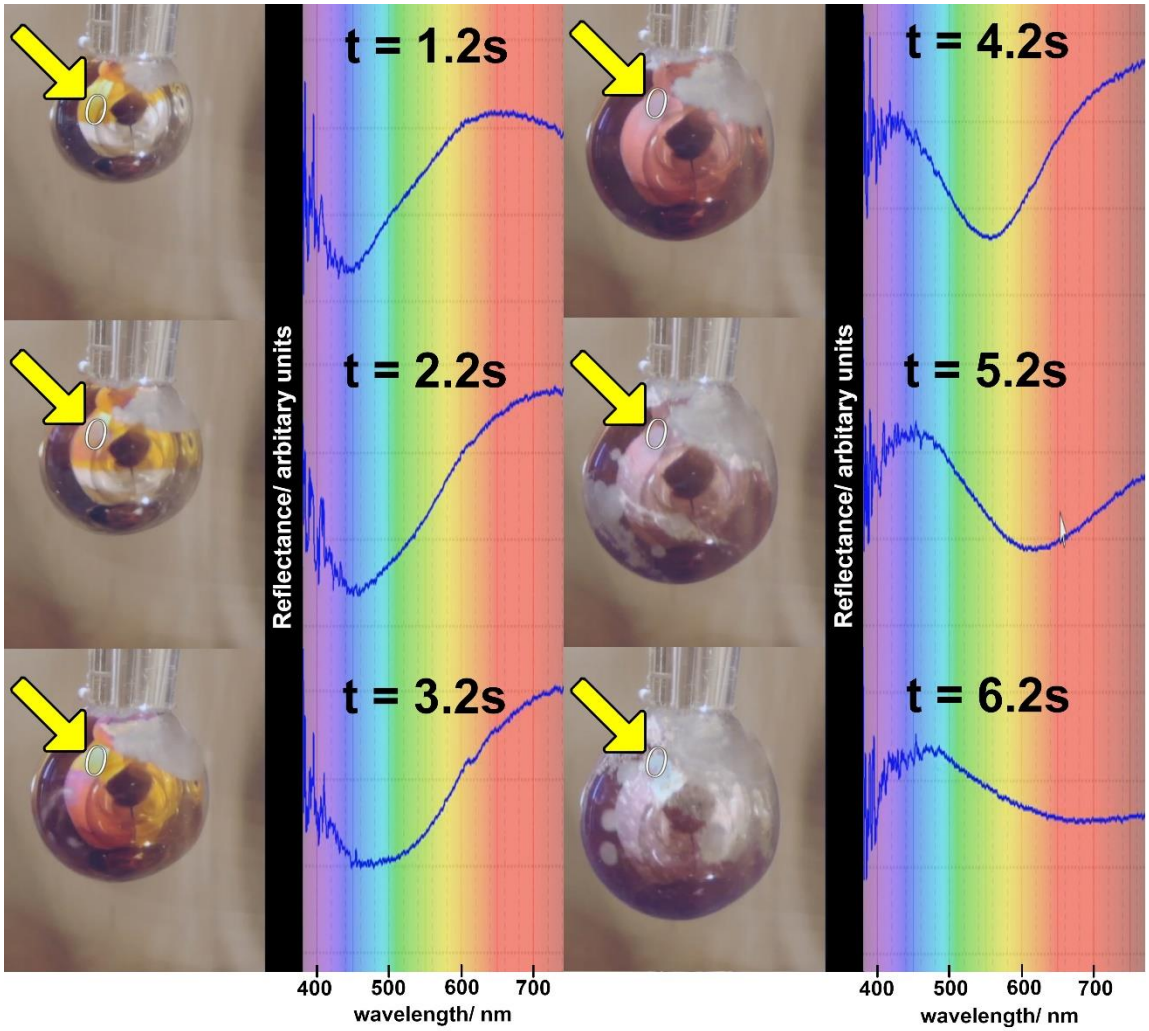
It would be exciting to have water with metallic properties; however, attempts to convert pure water from a perfect insulator into a metal by pressurizing the system remain in the realm of science fiction. This is because the estimated required pressure of 48 Mbar is an order of magnitude higher than what is accessible in the laboratory nowadays and may only exist in cores of large planets or stars. In this talk, we show that a metallic aqueous solution can be prepared by massively doping water by electrons liberated from alkali metals. Note that metallic solutions of liquid ammonia have been known for decades. However, it is a textbook knowledge that dissolution of alkali metals in water leads to an explosive chemical reaction, thus only low (sub-metallic) electron concentrations have been prepared so far. We have now found a way around the explosive chemistry by adsorbing water vapor at a pressure of about 10^{-4} mbar onto a train of liquid sodium-potassium alloy drops ejected from a nozzle into a vacuum chamber.



This leads to a formation of a transient gold-colored layer of water doped with ~5×10^{21} electrons/cm^3, the metallic character of which is demonstrated by a combination of optical reflection and synchrotron x-ray photoelectron spectroscopies.

ELECTROCHEMISTRY FOR ENERGY STORAGE: BATTERIES, HYDROGEN AND BEYOND

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The growing demand for energy, confronted with problems of fossil and nuclear fuels, highlight the need for renewable sources. Currently, ca. 13% of electricity in our country comes from renewables, in which the photovoltaics plays a significant role. With ca. 1600 hours of sunshine per year, the area of solar panels of ca. 10 m^2 per capita would cover the total consumption of electricity of households (without industry) in the Czech Republic. One of the problems of solar energy is the uneven dosage of sunlight during the day. Hence, electricity must be stored during overproduction and taken from these reserves when the sun is not shining. The second main reason for storage is grid stabilization, primarily for frequency regulation.

Li-ion batteries are currently used for backup of renewables and grid stabilization in big power reserve installations having up to 150 MW; yet the total share of global market is ca. 10 % only. (30 % corresponds to electromobiles and 60 % to consumer electronics). Boosting of energy density asks for novel concepts, such as lithium-air, where the cathode material is oxygen from the atmosphere. Another challenge is the Li-S battery [1] which is predicted to deliver 500-600 Wh/kg, i.e. ca. twice of the current batteries (Table 1). Such effective energy storage would even open a chance of using batteries for powering aircrafts in the near future.

Table 1: Energy capacity of selected materials or devices

Energy carrier	kWh/kg	kWh/L
Hydrogen (liquid 23 K)	33	2.4
Hydrogen (gas 20 MPa)	33	0.5
Gasoline	13	9
Coal	8	-
Lead-acid battery	0.03	0.09
Li-ion battery	0.25	0.5
Supercapacitor	0.005	0.01

Research on Li-ion batteries is in many practical and theoretical respects interfaced to investigation of an analogous system, viz. the electrochemical supercapacitor. Though the energy density of a supercap is relatively low (Table 1), its (dis)charging rate, quantified as the power density (of ca. 10 kW/kg) surpasses that of batteries of fuel cells significantly. For nanostructures, the effects of capacitive and faradaic storage of electrical charge are interrelated, due to the high surface area of nanocrystals. The proportion can be simply evaluated by mathematical deconvolution of cyclic voltammograms [2].

Hydrogen is regarded the "fuel of future". With the minimal environmental issues and high energy density, H_2 is competing the traditional fuels (Table 1). Hence, its use for sustainable

energetics, fuel-cell driven cars and other devices, as well as the hydrogen production by solar-driven water splitting is another challenge for science and technology in the 21st century. The history is traditionally (but wrongly) dated back to the Fujishima & Honda experiment in 1972 demonstrating water photo-electrolysis on photoexcited TiO_2 (rutile) single crystal electrode.

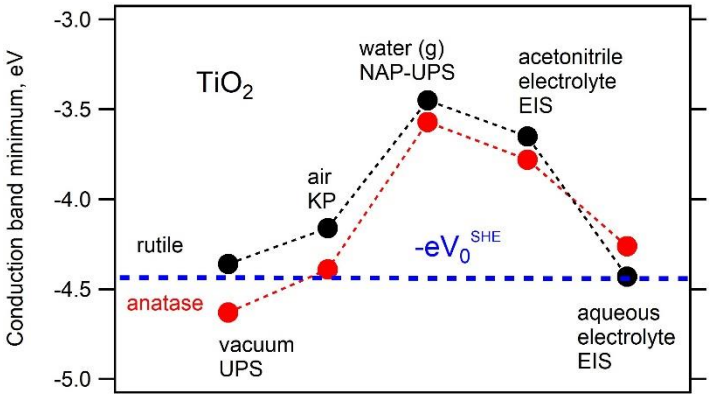

Figure 1: The position of conduction band minimum in TiO_2 single crystals (anatase or rutile) determined by various techniques: ultraviolet photoelectron spectroscopy (UPS), Kelvin probe (KP), near ambient-pressure UPS in water vapor (NAP-UPS) or electrochemical impedance spectroscopy (EIS). Blue line marks the energy level corresponding to the standard hydrogen electrode in the physical scale ($-eV_0^{SHE}$). Adapted from ref. [3]

This work is not without some technical objections pointing at the relatively low position of the conduction band minimum (CBM) in titania, which would hardly support the photoelectrolysis of water without the aid of external electrical or chemical bias [4]. We have recently revisited this problem by detailed electrochemical and other measurements of the CBM in well-defined TiO_2 single crystals [3], and confronted these data with earlier theoretical modelling [5]. Figure 1 summarizes the main findings, illustrating that the CBM position is dramatically dependent on the environment surrounding the semiconductors surface. Based on this knowledge, the traditional approach of relating the redox potentials of H^+/H_2 and O_2/O^{2-} couples to the CBM and VBM, respectively in a semiconductor photoelectrode, is quite problematic, despite it is frequently encountered in publications and textbooks dealing with water photoelectrolysis and related themes [3].

ACKNOWLEDGEMENT

The work was supported by the Grant Agency of the Czech Republic (grant No. 20-03564S).

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ELECTROCHEMICAL BIOSENSORS AS SMART TOOLS FOR CLINICAL DIAGNOSTICS

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Biosensors are currently an invaluable tool for the analysis of samples, especially in the medicine, pharmacology, clinical biochemistry, biotechnological and chemical processes, environmental analysis and food quality estimation. Biosensor applications are expanding rapidly due to the growing demand for fast and accurate quality or quantity control and detection of very low concentrations of substances.

Electrochemical biosensors are among the oldest and most widespread catalytic sensor devices and are based on conversion of biochemical processes, such as the reaction between the enzyme and the substrate, or the antigen-antibody interaction, on electrical signals (Fig. 1). The most common electrochemical sensors are enzymatic sensors, nanomaterial-based sensors, immunosensors, DNA sensors, and apatasensors. The main advantages of electrochemical sensors are simple construction of the measuring system, low costs, excellent sensitivity and specificity. In addition, these systems can be integrated into miniaturized analytical devices (lab on chip), which represent excellent analytical platforms for the point of care or on site analysis, which fully replace commercial laboratory instruments for in vitro diagnostics [1, 2].

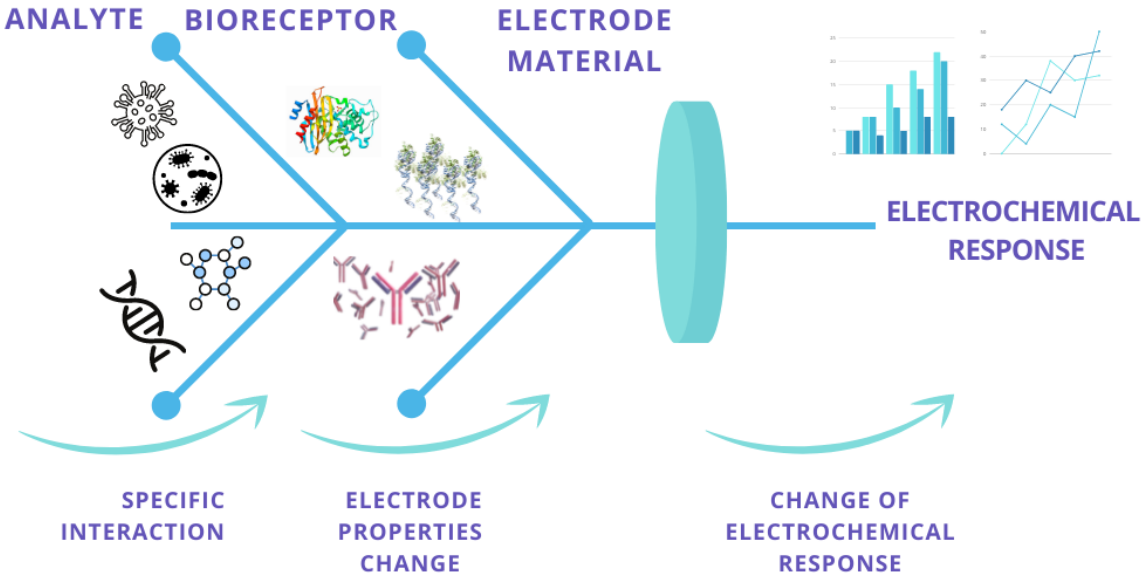

Figure: Principle of electrochemical biosensors.

Electrochemical biosensors have wider interest in the last decades than other analytical techniques such as chromatography, spectrophotometry, fluorescence, migration techniques and flow systems. The use of different nanomaterials in biosensing has enabled faster detection and its reproducibility in a much better way due to the unique properties of nanomaterials.

ACKNOWLEDGEMENT

The work has been supported by the Slovak Research and Development Agency (project APVV-PP-COVID-20-0036) and Visegrad fund (project No. 22020140).

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INVITED LECTURES

STRUCTURE-PROPERTY RELATIONSHIP IN MOLECULES WITH MULTIPLE CHARGE TRANSPORT PATHWAYS

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Molecules with multiple charge transport pathways have been studied in recent years by our group.[1–4] They contained tripodal anchoring groups, where the main purpose was to achieve more efficient contact geometry and charge transport coupling with an electrode surface. This contribution will discuss charge transport properties of molecules allowing also multiple charge transport pathways, but the main difference is in the fact that selected series of molecules are symmetrical and contain identical anchoring groups (pyridine) and central element with varying number of aliphatic bridges (from one to three), see Figure.
On these two series we will demonstrate peculiarities of a so-called quantum interference effect within the charge transport in the single molecule junctions.

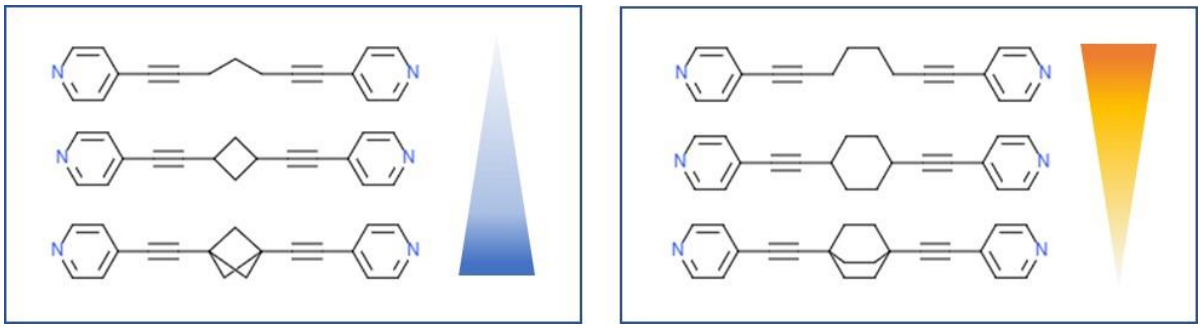

Figure: Schematic presentation of two series of molecules containing one, two or three CH_2 (left) and CH_2CH_2 (right) bridges.

The single molecule conductance was obtained by STM break junction technique in trimethylbenzene solvent. Density functional theory combined with the non-equilibrium Green's function formalism was used to understand charge transport properties and to provide most probable metal-molecule-metal junction geometries. As can be seen in the Figure we have employed two series of molecule. One contained an odd number of -CH_2- bridging units the other one contained an even number of -CH_2- units, namely -CH_2-CH_2- bridges. We have shown that the conductance is about one order of magnitude higher for molecules with -CH_2- bridging units compared to -CH_2-CH_2- bridges. This observation is in accord with recent studies on σ-

bonded silanes with increasing backbone length.[5-7] The single molecule conductance in CH_2-containing bridges increases with increasing number of bridging units (one to three). The opposite trend was observed experimentally for molecular bridges with -CH_2-CH_2- units, see Figure above. Quantum chemical calculations were able to reproduce the experimental results and provided the understanding of essential features of the electron transport in these systems. Clearly the single molecule conductance values do not follow the Kirchhoff's law of the electric circuit. We have observed experimentally an increase of thirty percent by addition of the second -CH_2- conductance channel and 2.3 times higher conductance for three such channels. Even though the molecular junction length decreases for a series of molecules with -CH_2CH_2- bridges the conductance is attenuated by thirty percent in a molecule with two bridges and by an additional ten percent for molecule with three such bridges. What is more important, we confirmed the odd-even effect by showing that the constructive quantum interference can be changed to the destructive one just by prolonging the σ-bonded conduction channels by one CH_2 group.

ACKNOWLEDGEMENT

This work has been supported by the Czech Science Foundation (21-13458S) and the Czech Academy of Sciences (RVO: 61388955).

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REDOX PHOTOCHEMISTRY: ELECTROCHEMICAL STUDIES OF PHOTOINDUCED ELECTRON TRANSFER

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Photoinduced electron transfer is a key process in photoredox catalysis, a field that has experienced a rapid growth in the last decades. Single electron transfer between a suitable electron donor and the excited state of a photocatalyst generates a pair of radical ions that either recombine or undergo a rapid chemical transformation (e. g., deprotonation, dehalogenation, C-C bond cleavage).[1] The energetics of the photoinduced electron transfer can be estimated from the ground state redox potential of the substrates and the photocatalyst and from the energy of the catalytically active excited state. While most photoredox catalyst have known redox properties in the excited state, other molecules forming stable radical ions, namely redox switches[2] and triarylamines,[3] are underexplored in terms of photoinduced electron transfer. We combine electrochemical studies with spectroelectrochemistry and spectroscopy to investigate the redox properties of excited states of stable radical ions relevant for molecular electronics and organic photovoltaics.

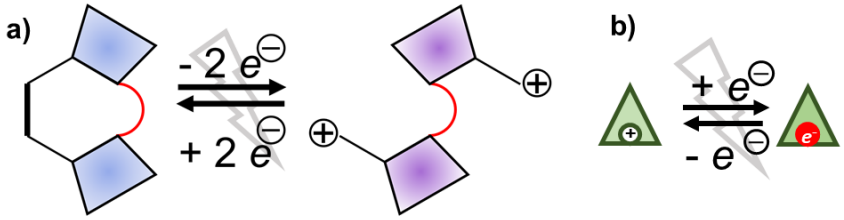

Figure: Schematic representation of light-driven electron transfer between redox switches (a) and triarylamines (b).

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ON THE ROLE OF UV-VIS AND IR SPECTROELECTROCHEMISTRY IN DETERMINATION OF REDOX MECHANISM OF ORGANIC COMPOUNDS

Romana SOKOLOVA[1*], Ilaria DEGANO[2], Jan FIEDLER[1], Jana KOCABOVA[1]

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The electrochemical methods can significantly contribute to the understanding of processes, where electron transfer reactions play an important role. The formation of radicals during oxidation or reduction, reaction mechanisms involving electron and proton transfers, the presence of short living intermediates, all contribute to the comprehension of drugs activities, their mode of action and determination of their metabolites. Therefore, the oxidation and reduction mechanism involving complexed reaction schemes should be determined. The cyclic voltammograms recorded in different conditions (e.g. at different pH values) gives unique information about the reaction scheme. This is based on the knowledge that chemical reactions directly affect the concentration of the electroactive species available at the electrode surface. In particular, the analysis of oxidation waves allows us to distinguish between reaction schemes entailing EC (Electron transfer followed by Chemical reaction), CE (the Chemical reaction precedes the Electron transfer), ECE, EE, ECEC etc., as well as disclosing oxidation or reduction coupling with catalytic processes [1]. For instance, the shift of peak potential in solutions at different pH values means that protons participate in redox process [2,3].

The complete elucidation of the oxidation or reduction mechanism requires the use of analytical separation techniques and on-line spectroelectrochemical methods [4,5]. *In-situ* UV-Vis spectroelectrochemistry is an efficient technique giving information about the changes in absorption spectra during electrolysis at controlled potential or in cyclo-voltammetric regime (Figure). Spectroelectrochemical measurements are performed in optically transparent thin layer electrochemical cells [6]. Additionally, *in-situ* FTIR spectroelectrochemistry can characterize reactions occurring at the electrode surfaces by monitoring the change in absorbance of vibrations belonging directly to the functional groups participating in the redox process. Based on the principles mentioned above, we recently disclosed the difference in the oxidation mechanisms of flavonols (as quercetin, fisetin, rhamnazin, and rhamnetin) and flavanones (taxifolin) or flavons (luteolin) [7,8].

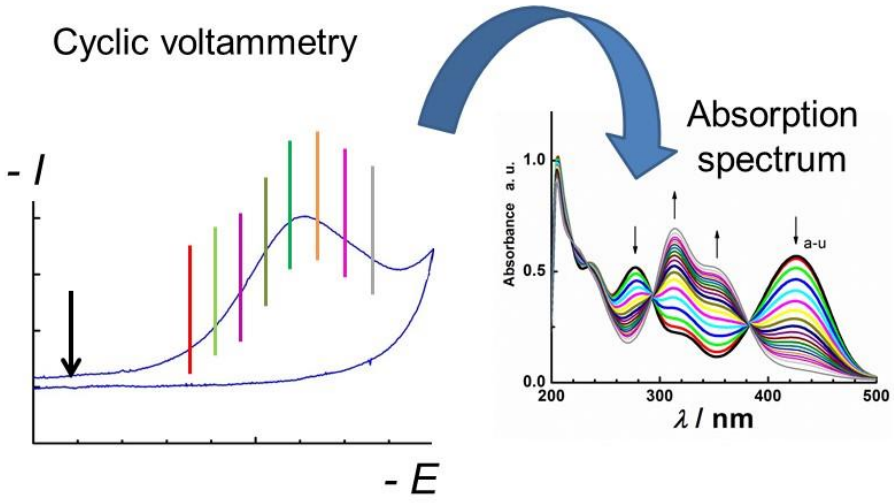

Figure: UV-Vis spectroelectrochemistry

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PROTEIN DYNAMICS SEEN BY FLUORESCENCE: STUDY ON HALOALKANE-DEHALOGENASE ENZYME

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The protein dynamics is believed to profoundly affect their function. However, only a few approaches for its monitoring within the relevant protein regions are available. Here we describe two general methods for site-specific analysis of the extent of mobility in enzyme Haloalkane Dehalogenase. The first approach is based on recording „time dependent fluorescence shift“ (TDFS) placing the dye in the tunnel mouth of this enzyme[1]. Furthermore, the „gating“ dynamics of the enzymes can be traced by following the photoinduced electron transfer (PET) between the selected tryprophan and properly positioned fluorescence dye[2]. The dynamics monitored within the biologically relevant regions of the dehalogenase enzymes is then compared with their enzyme kinetics of various mutants, which can bring the deeper insight into the functioning of these enzymes.

ACKNOWLEDGEMENT

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ORAL PRESENTATIONS

MONITORING THE INFLUENCE OF SWEAT MATRIX ON INTERACTION OF SELECTED NANOPARTICLES WITH DNA

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Nanomaterials are substances with specific properties that are widely used in medicine and cosmetics. Due to their small size, they are able to penetrate cells and cause significant damage or degradation by interaction with various biomacromolecules [1]. The use of TiO_2 and Al_2O_3 nanoparticles (NPs) in cosmetics poses a health risk. Their exposure to UV radiation cause cellular toxicity, DNA damage and induction of carcinogenesis. Rapid analysis of the given risks using simple, fast, inexpensive and compact devices such as DNA biosensors is important [2,3].

Electrochemical detection of interactions between TiO_2 and Al_2O_3 NPs with DNA biomacromolecule in the environment of real sweat (RSw) and synthetic sweat (SSw) matrices was investigated by cyclic voltammetry (CV) and differential pulse voltammetry (DPV) using DNA biosensor or biosensing approach. For Al_2O_3 NPs, deeper DNA damage of the biosensor was observed with increasing incubation time. From the CV records of the effect of UV radiation on the interaction of the SSw and RSw matrix with Al_2O_3 and TiO_2 NPs, the largest damage of DNA biosensor was induced by Al_2O_3 NPs in the SSw environment after 15 minutes of irradiation. Significant DNA damage in the SSw environment after exposure to UV radiation for both types of nanoparticles was recorded by the biosensing method using DPV.

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ELECTROCHEMICAL BEHAVIOUR OF OLIGONUCLEOTIDES ADSORBED ON PYROLYTIC GRAPHITE ELECTRODE

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Electrochemical oxidation of DNA adsorbed on basal plane pyrolytic graphite electrode (bPGE) is known since 1978.[1] Even though the first cathodic reduction of purine at bPGE was observed by Dryhurst and Elving in 1969 [2], label-free detection of canonical DNA bases, uracil, and 5-methylcytosine at bPGE in oligonucleotides were presented by Spacek et al in 2017. A broad range of applicable potentials (from −2.0 to +1.6 V in acetate buffer pH 5) significantly extended the possible application of bPGE in DNA electrochemistry.[3] In this study, we have focused on deeper study of electrochemical behavior of selected sequences adsorbed on bPGE, with the aim to distinguish single-stranded DNA from guanine reach sequences, which are able to fold into four-stranded noncanonical secondary structures called G-quadruplexes (G4s). G4s were initially considered a structural curiosity, but recent evidence suggests their involvement in key genome functions such as transcription, replication, genome stability, and epigenetic regulation, together with numerous connections to cancer biology.[4] Thus, an influence of ODN sample composition (presence of Li^+/K^+ and ionic strength) from which ODN is being adsorbed on bPGE, adsorption time, ODN concentration, and the composition of the electrolyte has been observed. Obtained results will be presented.

ACKNOWLEDGEMENT

The work has been supported by the SYMBIT project reg. no. CZ.02.1.01/0.0/0.0/15_003/0000477 financed from the European Regional Development Founds.

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PHOTOELECTROCHEMICAL NERVE CELL REGULATION WITH LIGHT

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A great demand exists for minimally-invasive neuromodulation technologies to enable next-generation bioelectronic medicine. We report on our developments of ultrathin (opto)electronic devices for neurostimulation. These devices rely on far red/near infrared irradiation in the tissue transparency window to actuate organic semiconductor components. Our flagship technology is the organic electrolytic photocapacitor (OEPC) – a device that mimics biphasic current-pulse neurostimulation and thus transduces an optical signal into directly-evoked action potentials in neurons. We will discuss chronic implants capable of stimulating peripheral nerves as well as the brain, when actuated from outside of the body. We believe that the combination of deep red light and ultrathin photovoltaic devices can account for a new paradigm in wireless bioelectronic medicine.

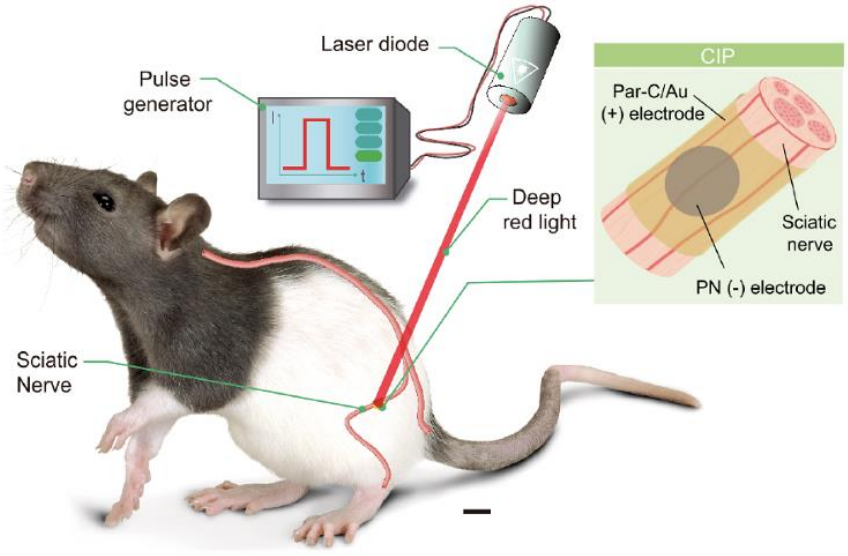

Figure 1: Organic electrolytic photocapacitors (OEPCs) wirelessly stimulate the sciatic nerve *in vivo*. Scale bar 1 cm. The inset details how a chronically-implantable photocapacitor (CIP) cuff is placed around the nerve. Following implantation, deep-red light penetrates through skin, fat, and muscle tissues to reach the OEPC, located at a depth of roughly 10 mm.

ACKNOWLEDGEMENT

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ESTABLISHING OF ARBRE – ASSOCIATION OF RESOURCES FOR BIOPHYSICAL RESEARCH IN EUROPE

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Molecular-scale biophysics is a dynamic interdisciplinary field that aims to study biological macromolecules and assemblies as a whole, at an intermediate level between atomic-resolution structural descriptions and cellular-level observations with significant applications in biomedicine and drug discovery. There has been established numerous biophysical core facilities and other laboratories enabling users of various background to use the advanced instrumentation. Since the development of science is enormous over last decades, the collaboration and sharing of know how between such facilities is necessary in order to keep and develop the state of the art technologies.

In 2014, the ARBRE-MOBIEU network was initiated, aiming to seed a large-scale pan-European interdisciplinary clustering, allowing to ally and synergize the power of spectroscopic, hydrodynamic, real-time microfluidic, thermodynamic and single-molecule approaches [1]. In its early years, the network was supported by a European COST action, resulting in involvement of several dozens of laboratories throughout Europe. In 2021, based on the established contacts and collaborations, the initiative has been turned into a scientific society ARBRE (Association of Resources for Biophysical Research in Europe).

The main objectives of the society are to: i) create an optimal environment for the development of innovative integrative biophysical approaches; ii) disseminate knowledge, e.g. through the organization of workshops and training schools; iii) facilitate the transnational access to instrumentation and expertise for a wide user community; iv) provide a platform for scientists to establish early contacts with instrument developers. The users can already benefit from several outcomes, such as development of standards for interaction techniques [2], establishing of standard operating procedures (SOP's) [3] or formulating recommendations for protein quality control [4] and stability assessment [5].

You can visit the association web pages (https://arbre-mobieu.eu/) for more information or contact its representatives directly.

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CONFORMATIONAL CHANGES UPON PHOSPHORYLATION OF PROLINE RICH REGION OF TAU(210-240) FRAGMENT USING MOLECULAR DYNAMIC SIMULATIONS AND NMR SPECTROSCOPY

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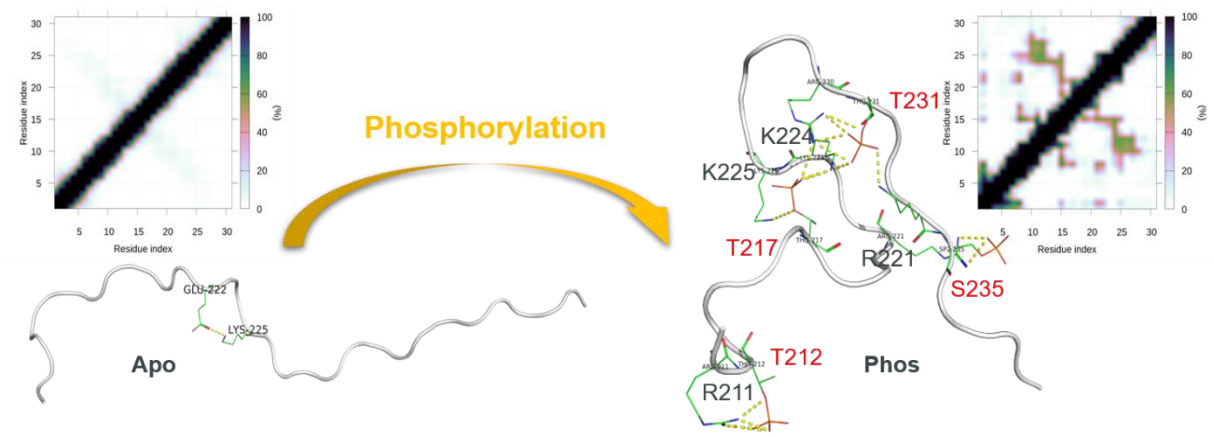
The conformational and dynamic changes of protein interaction regulated by posttranslational modifications such as phosphorylation of intrinsically disordered proteins (IDPs), remains challenging to elucidate. Tau, which is a well-known IDP and its phosphorylation is of particular interest because hyperphosphorylated Tau is found in neurofibrillary tangles of Alzheimer's disease. Here we decided to study fragment of Tau(210-240) and its conformation changes upon phosphorylation. This proline-rich fragment contains recognition interaction sites for several proteins such as as 14-3-3s and SH3 domain of BIN1.

Based on our previous validation of different force-field parameters for IDP regions when comparing with available NMR properties [1] we have decided to apply two different force field parameters (AMBER99SB-ILDN and CHARMM36m) with TIP4PD water model. Microsecond time scale molecular dynamic simulation studies were performed for apo and phosphorylated Tau fragment (210-240) at three different temperatures (278 K, 298 K and 310 K). The obtained molecular dynamics trajectories were used to predict measurable parameters, including radii of gyration, NMR chemical shifts and 3J couplings of their individual residues. Afterwards the structural ensembles having the best agreement with experimental NMR data have been selected and extended. Analysis of the most reliable structural ensembles revealed that phosphorylation of Tau fragment significantly induces the conformational changes leading to the bent conformer and increases its compactness. The revealed structural alteration provide detailed understanding about modified interaction properties of Tau (210-240) fragment after its phosphorylation.



ACKNOWLEDGEMENT

The work has been supported by MEYS-CR Inter-Excellence Inter-Action grant scheme (no. LTAUSA18168) and by the Czech Science Foundation (no. GF20-05789L). Computational resources were supplied by (metacentrum and IT4Innovations National Supercomputing Center) the project "e-Infrastruktura CZ" (e-INFRA LM2018140) provided within the program Projects of Large Research, Development and Innovations Infrastructures. Mr. Krishnendu Bera is also supported by Brno Ph.D. Talent Scholarship – funded by the Brno City Municipality, Brno, Czech Republic.

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COVALENT MODIFICATION OF A HEME-LIGATING CYSTEINE IS KEY TO CATALYSIS IN THIOSULFATE HYDROGENASES

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The unusual His/Cys^- axial ligation of the active site heme of the diheme bacterial sulfur cycle enzyme thiosulfate dehydrogenase gives it an E_M = -185 mV (vs. SHE)[1]. Given that the thiosulfate/tetrathionate couple has an E_M = +198 mV[2], this value does not easily explain the driving force for the efficient Thiosulfate Oxidation catalyzed by this enzyme in pathogenic bacterium *Campylobacter jejuni*[3]. Sulfite, an analog of the native substrate Thiosulfate, was used to prepare a long-lived modified form of this enzyme where the active site Cys^- becomes Cys-SO_3^-as detected by mass spectrometry. Magneto optical spectroscopy and cyclic voltammetry on indium tin oxide electrodes were then used to characterize this species, revealing displacement of Cys-SO_3^- as an active site ligand by water and consequent +200 mV increase in heme E_M. This species is analogous to the thiosulfate reacted form of the enzyme[4], confirming this as a key catalytic intermediate and rationalizing the natural activity of this enzyme.

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STUDY OF AMINOPHYLLINE EFFECT ON RAT CARDIOMYOCITES USING MULTIELECTRODE ARRAY

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The bronchodilators (BD) are used by patients with a low lung's airflow, asthma and chronic obstructive pulmonary disease are the usual reasons for the use of these medication. However, long-term overuse of bronchodilator combinations may cause cardiac arrhythmias, tachycardia, and chronic heart failure, that may lead to the death. Myocardial response to BD and its combinations has not been fully described. Thus, the study of the biophysical and biochemical effect of bronchodilators is a promising task in pharmacology studies. Microelectrode array (MEA) method was employed to characterize field potential of the cardiac cells in a real time. The MEA platform, consisting of a two-dimensional array of microelectrodes, was developed for collecting spatiotemporal bioelectrical signals, to describe cellular potential and other parameters (e.g., amplitude, duration, and firing rate). Field potential duration (FPD), the time from repolarization to depolarization measured via MEA, correlates with action potential duration [1]. Moreover, the indicators of FPD correspond to changes in ECG parameters *in vivo* that makes this method most relevant for cardiac studies [2].

Line of rat cardiomyocytes HL-1 was used to study cardiotoxicity of salbutamol and aminophylline bronchodilators. The field potential of the HL-1 cells was measured using the MEA2100-mini-60 (Multi Channel Systems, Germany). The measurements were carried out in a Claycomb medium at 37 °C for 3 minutes. The data was analyzed using Multi Channel Analyzer and an in-house Python script.

Aminophylline has been tested in a concentration range of 8-512 μmol. At a concentration of 128 μmol, the average frequency (beat rate) increased by ~8%, at concentrations of 256 and 512 μmol, the effect was ~13% compared to control. Using chi-square test presence of arrhythmic effect was shown on R-R distances data (Fig.1). Aminophylline had no effect on the amplitude of the field potential. Thus, the cardiotoxic effect of aminophylline and its features are shown. Further research on bronchodilators and their combined effect is needed.

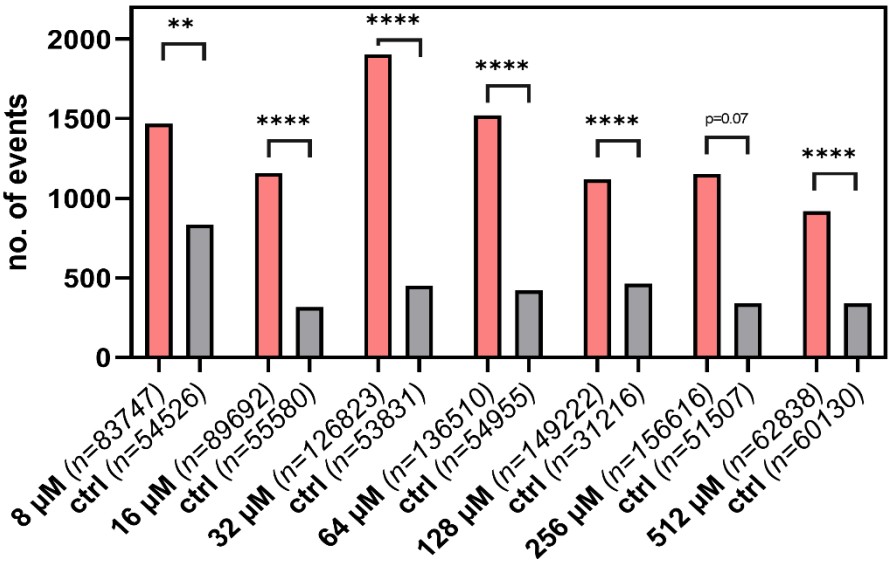

Figure 1: Number of arrhythmic events in control (ctrl) and with various concentrations of aminophylline (8-512 µM).

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ELECTROCHEMISTRY OF PENTASUBSTITUTED PHOSPHOLES – PROMISING MATERIAL FOR ORGANIC ELECTRONICS

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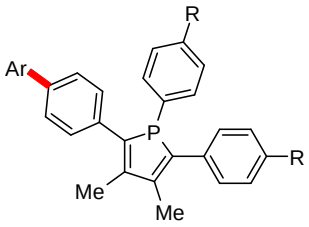
Phospholes are five-membered heterocycles similar like pyrrol, thiophene or furan. There exist also fused, planar derivatives of phosphole, but our study is focused on pentasubstituted phospholes, where positions 1, 2 and 5 are substituted by aromates enabling to introduce extended π-systems.

Phospholes are non-planar rings with tricoordinated P having a non-bonding el. pair. Among the others five-membered heterocycles, its aromaticity is the lowest - it has rather diene character with very low participation of the phosphorus atom.

These compounds have unusual steric and chemical properties and represent promising material for organic electronics (org. solar cells, fluorescent probe, OLEDs etc.). Therefore detailed physico-chemical, namely electrochemical investigation is necessary for their new applications.



From the synthetic point of view, new methodology based on the Suzuki cross-coupling reaction was developed [1]. The reaction enabled efficient introduction of electron-rich, electron-poor and electron-neutral aromatic or heteroaromatic substituents with various extent of π-delocalization of the exocyclic π-conjugated substituents [2].

For investigations, a series of 17 compounds was selected, where various comparisons resulted in formulation of more general rules enabling "tuning of properties and predicting of behavior. The substitution in positions 1, 2 and 5 significantly affects the properties of phospholes for their applications in material chemistry. The combination of phosphole core and various aromates (pyrene, thiophene, diphenylamino group etc.) resulted in the study of the optical properties where increasing degree of phosphole conjugation induces bathochromic shift of the absorption bands in the UV/vis spectra. The most important electrochemical data (taken from polarography, GC-RDE and cyclic voltammetry) are the first oxidation ($E_{ox}1$) and the first

reduction ($E_{red}1$) potentials which correlate qualitatively with HOMO and LUMO energies, respectively and the potential difference ($E_{ox}1$)-($E_{red}1$) is proportional to the HOMO-LUMO gap. Generally, from the electrochemical point of view, less positive $E_{ox}1$ and/or less negative $E_{red}1$, that means lower difference between these potentials should correspond to the bathochromic shift pointing to a more delocalized system. This effect, however, is strongly affected by molecular geometry, that means by dihedral angles interconnecting the present aromates [2]. The out-laying values or discrepancies within a series of similar compounds are very informative because they point to a different electron distribution within the molecule, that means different redox center and, thus, different mechanism.

ACNOWLEDGEMENT

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ADVANCED BIOPHYSICAL METHODS FOR CHARACTERIZATION OF BIOSAMPLES

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Nanotechnology is rapidly developing part of a modern science. Nanobiotechnology is expanding the potential by incorporation of the biomolecules and other living objects such as cells and their clusters. Our laboratory is performing research in the field of imaging of biomolecules [1], mapping the elastic properties of cells and their clusters [2], and characterization contractile properties of cardiomyocytes and their clusters [3].
The ongoing research will be presented, however, the new equipment of the core facility will be described as well.

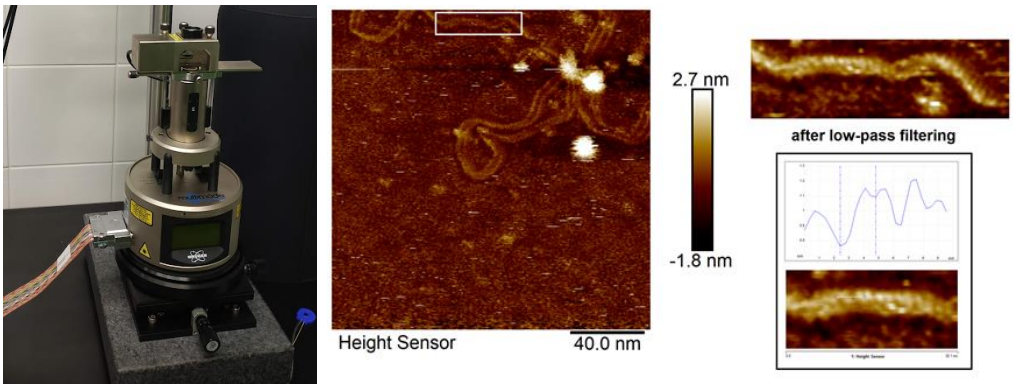

Figure: New BioAFM Bruker MultiMode 8HR (left), maximum resolution illustrated on the right, where the double helix of ds plasmid structure is shown.

The laboratory's flagship is the large AFM microscope JPK NanoWizard 4XP installed on a Leica DMi8 optical microscope with a fluorescence module. Both microscopes can operate simultaneously in the so-called directoverlay mode, thus combining AFM and optical microscopy abilities. Moreover, the new AFM microscope offers an extended operation range, compared to the previous model (NW 3) operated in our laboratory from 2014. The maximal range of operation is now extended to the 200x200x200 µm by hybrid stage, an essential factor for working with rugged samples such as bone sections or plant samples. The NanoWizard 4XP AFM microscope is not only an imaging tool, however, helps to map elastic properties of various samples with nanometer resolution. One of the main advantages is the ability to work in semi-physiological conditions.

The new AFM device is equipped with a compelling module, the so-called FluidFM. This technology combines the precise nanomechanical indentation ability of the atomic force

microscope with microfluidics. Microchannel inside the cantilever changes this tool into the nano-pipette, able to aspirate and/or deliver extremely low volumes. This feature can be used when injecting or removing small volumes from individual cells. Using stiffer cantilevers, the system can investigate cell adhesion on new types of implant materials.
Keeping on the cutting-edge current AFM technology, the new generation of the MultiMode AFM microscope, version 8HR, was built for imaging with the maximum resolution that current commercial setups allow. This AFM setup will help the structural biologist image the biomolecules (DNA, proteins, molecular complexes) on a single molecular level.
A multielectrode array (MEA) is a grid of tightly spaced microscopic electrodes embedded in the bottom of each well in a multi-well MEA plate. Cells, such as cardiomyocytes or neurons, electrically active, can be cultured over the electrodes creating a cohesive network. The functional behavior or electrical activity of this network can be recorded. These action potentials are recorded extracellularly and are known as field potentials. MEA device can be used to record spontaneous activity from hiPSC-derived neuronal cells upon differentiation and maturity, cell cultures for disease modeling, drug screening, or toxicology studies. Moreover, the MEA can be simultaneously connected with an AFM microscope, thus studying mechanoelectrical feedback of cardiac cells, tightly connected with some heart pathologies, such as catecholaminergic polymorphic ventricular tachycardia (CPVT).

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INTERPRETING IMPEDANCE SPECTROSCOPY: MORE IS SOMETIMES NOT BETTER

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The method of electrical impedance spectroscopy is based on an experiment in which a frequency dependent resistance, measured over a broad range of frequencies is collected. Thereafter, ideally, the collected data are interpreted in some manner with the goal to provide information about chemistry of physics of the studied object. The established approach of data interpretation is to treat the circuit as if it were an electric circuit consisting of discrete elements such as resistors, capacitors and inductors. To fit other behavior not mimicked by these fundamental components, more complex elements are introduced, such as the Warburg element, describing diffusion.

There are some fundamental rules the collected data need to fulfill so that they can be interpreted using the equivalent circuits. They have to be:

- Ergodic (stable)
- Deterministic
- Causal (conservation of mass, charge, etc.)
- Linear
- Finite (avoid singularities)

There are also some practical rules devised to make sense of the meaning of the individual elements in the equivalent circuit. A good advice to follow is to have some basic understanding of the physical or chemical meaning of each element. Assigning resistance as a series solution resistance or charge transfer resistance and capacitance as the double-layer capacitance is more trivial. But sometimes a meaningful assignment can become challenging.

There are also good practice rules that one should follow. While it is desirable to have good confluence between the model and the experiment, one should not employ excessive number of elements in the model. With many elements it will be possible to achieve a perfect fit for each collected data point, but that would not reflect reality. Likewise, too few elements are not desirable either. However, there is a situation when a fairly obvious feature in the impedance response calls for a circuit element, but it may be an artifact stemming from the way how the instrument operates and the element may be disregarded.

In some early electrochemical work in which we were using a three-electrode setup with a reference electrode consisting of a hydrogen electrode connected to the cell via a Luggin capillary, we discovered an interesting phenomena [1]. The standard operating practice for cyclic voltammetry was to keep the ground glass stopcock on the Luggin capillary closed to avoid acid leakage. The concentrated sulfuric acid layer in the wet ground glass joint should be conductive enough. While there was never noted any issue in voltammetry, we realized that impedance response with the closed stopcock was quite different from the open stopcock case. Specifically, with the stopcock closed, there sometimes appeared one more feature at high

frequencies in the Nyquist plot. This behavior was eventually attributed to an increased resistance on the input to the reference electrode part of the circuitry.

To understand this phenomenon, we designed an experiment using a Solartron 1255 impedance analyzer and Solartron 1286 interface (potentiostat) with a four-electrode setup. In the experiment we studied a response of the Solartron test module 12861 ECI, while adding resistors in series on the inputs for the reference electrodes to the potentiostat. Fig. 1, upper part, shows, with the exception of the added resistor in the red rectangle the circuitry inside the 12861 ECI test module. The impedance response of the connected circuit should be the one of the components between the Reference electrode 1 and Reference electrode 2 (part in the green rectangle) jacks. This is the circuit with a single resistor (1.8 kΩ in series) with two parallel RC circuits with two time constants in series. On a Nyquist plot this circuit gives a two semicircle response, offset from the graph origin by 1.8 kΩ. That is approximately the response seen at the bottom of Fig. 1, highlighted in yellow. Adding various resistors between the reference electrodes inputs of the potentiostat, a series of responses with additional features at the high frequency ends were obtained. Fig. 1 show the result of adding a resistor 33 kΩ on the input of the Reference electrode 1, and leaving connection to Reference electrode 2 as normally intended. The resulting Nyquist plot is the full red line in Fig. 1, i.e., the two expected semicircles, plus an additional partial semicircle (red arrow). Similar findings were also reported by Anderson and Bühlmann [2] and Veal et al. [3].

Modeling realistic inputs of a potentiostat with high input impedance of 10^{10} Ω and capacitance 5 pF using Electronic Workbench we demonstrated that the high frequency response leading to an additional circuit element is inherently due to the existing instrument input time constant and is not an artifact of a particular potentiostat or a lack of its calibration.

In further analysis of the data using nonlinear least square fit we were able to show that the artifacts can be modelled (as a semicircle would be) by a parallel RC circuit, albeit resulting sometimes in need of a negative value of the series resistor. Once the effect of the input resistance is understood, data fitting can be reasonably simplified by judicious omission of the data at higher frequencies.

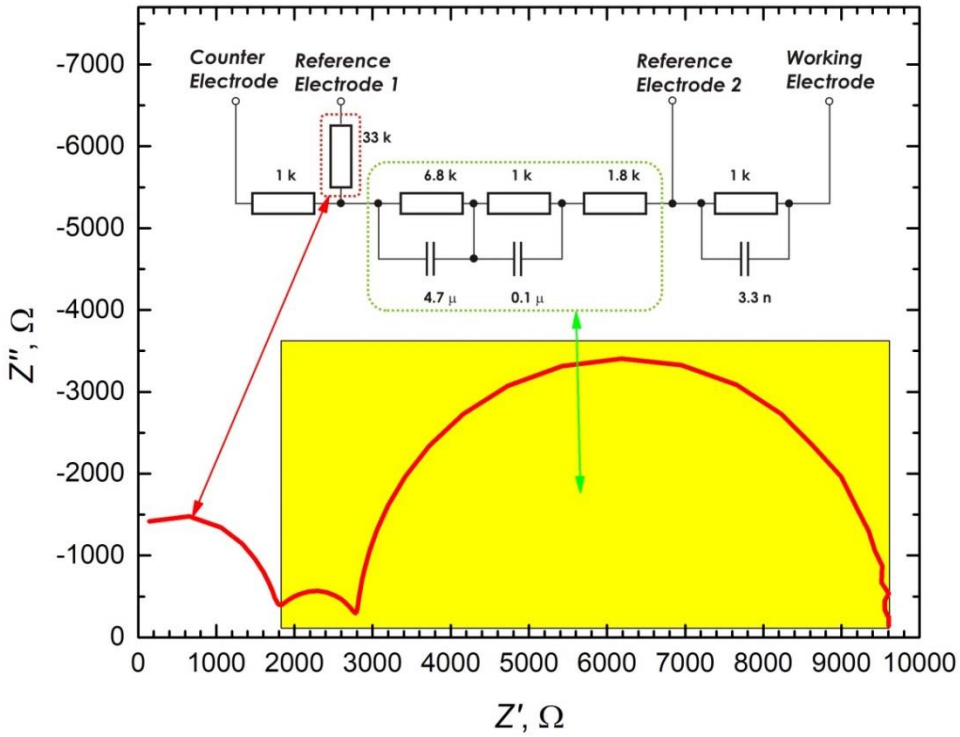

Figure 1: Upper part: Circuit connected to a potentiostat leading to an impedance response in the Nyquist plot depiction (red line, bottom part). The intended response of the circuit is in the yellow rectangle emphasis (green arrow). The real response, full red curve with additional semicircle (red arrow) is caused by adding a 33 kΩ resistor in series with input to Reference electrode 1.

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COMPETITIVE PRESENTATIONS OF THE YOUNG SCIENTISTS

DETECTION OF AUTOANTIBODIES AGAINST ABERRANT GLYCANS PRESENT IN CANCER DISEASES

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The presence of aberrant glycans (Tn antigen, sTn antigen, T antigen) has been demonstrated in many types of cancer (prostate, stomach, colon, lungs, esophagus…), to which the immune system responds by producing antibodies circulating in the blood.
Using biosensors, we are able to measure the level of antibodies in the sample. In this work we focused on optimizing the conditions for the preparation of a glycan biosensor sensitive to anti-Tn antibody and lectin DBA. The main electrochemical method for determining glycan-protein interactions was differential pulse voltammetry (DPV). The developed biosensor detected an analyte with high selectivity and sensitivity up to the atomolar level. We monitored the electrochemical behavior on variously treated surfaces of screen-printed graphene electrodes by cyclic voltammetry (CV). Subsequently, we detected antibodies by creating a more commonly used test – the standard and modified ELISA method, which is a clinically used test.

ACKNOWLEDGEMENT

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THE EXCEPTIONAL PENCIL LEADS: TWO APPLICATIONS OF POLYMER GRAPHITE SHARP TIPS

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Polymer graphite (PG) is broad range of micro- and/or nano-composites containing graphite flakes and various polymer-based binding agents. Although marketed mainly as refills for mechanical pencils, their high conductivity and immunity against surface contamination, make them a highly suitable material for electrode manufacturing in general. For the fabrication of sharp PG tips, three different methods were developed and tested: mechanical sharping, focused ion beam (FIB) milling and electrochemical etching. Several commercially available PG leads were selected for the study.

We chose to examine two different application fields, where PG tips can be employed, as very affordable Scanning Probe Microscopy probes for fast topology scans and as tunable cold field emitters. The performance of PG sharp tips was evaluated in Scanning Probe Microscope (SPM) or in Field Electron Microscope (FEM). Additionally, Raman spectroscopy, Scanning Electron Microscopy, Energy-dispersive X-ray spectroscopy and X-ray photoelectron spectroscopy measurements were carried out to investigate connections between their performance and their material composition, which differs across manufacturers. Ultimately, each PG sample was analyzed by the means of chemical dilution using acids and an additional analysis of ICP-MS for quantitative determination of 26. When used as cold field emitters, the charge transport and total emission current stability of PG tips were analyzed using noise spectroscopy [1].

The SPM probes made of PG proved to be reliable for routine characterization of the samples in air, with low resolution of surface features. The best results were achieved with electrochemical etching and FIB milling; these methods allowed fabrication of probes for imaging of fine nanoscale surface features.

The field emission behavior of sharp PG tips was in a way similar to a conventional single tungsten tip covered with conducting nanoparticles, acting as multiple small-diameter electron sources on single tip. This effect was attributed to sharp edges of protruding graphite flakes. Micrograph of electrochemically etched PG tip and its Pattern Image illustrating several emission spots from surface of the tip are in the Figure 1. Field emitter performance analysis was based on current–voltage data measured in the FEM using a recently developed methodology based on Forbes' Field Emission Orthodoxy Test [2,3].

PG tips from different manufacturers showed varying performance in SPM and FEM, which was directly linked to their greatly dissimilar material composition. For PG cold field emitters, we proposed simple electron trap model, which quantitatively describes the effects of admixture atoms on performance of PG tip field emitter.

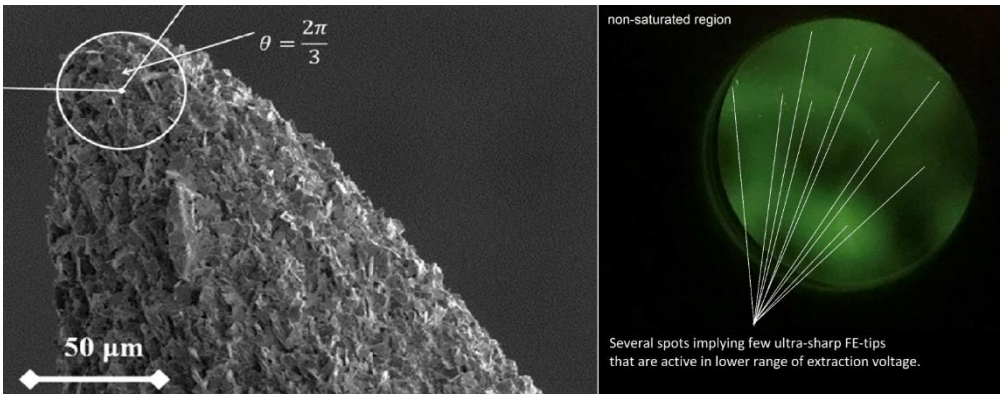

Figure 1: Micrograph (left) showing PG tip apex with several graphite flakes pointing upwards, apex tip radius calculation is illustrated. Field Emission Microscope Pattern Image for non-saturated emission current region showing multiple emission spots (right), diameter of the scintillator plane here is 31.5 mm.

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EXPLORATION OF TRANSIENT SECONDARY STRUCTURAL MOTIFS WITHIN MICROTUBULE ASSOCIATED PROTEIN 2N4R TAU

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Pathological conformational changes of microtubule-associated protein tau are often connected with many neurodegenerative diseases (eg., Alzheimer's disease). Due to the lack of tertiary structure tau belongs to the group of intrinsically disordered proteins (IDPs). The widely used techniques Cryo-EM and X-ray crystallography are not sufficient for structural characterization of these IDPs, on the other hand, nuclear magnetic resonance (NMR) can provide structural information at single residue resolution.

For structural characterization, we employed 5D ^{13}C-directly detected multidimensional NMR experiments for the backbone assignment of tau, because of tau's IDP character as well as the high content of prolines in its primary structure.[1] With this approach, we have successfully assigned 99,7 % of the protein backbone including all problematic prolines, and determined the secondary structure propensity at single residue resolution. We also performed side-chain assignment of all residues with backbone assignment and investigated X-Pro peptide bond conformations within tau. In the future, we plan to use this assignment to study long-range contacts within tau as well as its phosphorylation kinetics. We also want to investigate conformational changes of tau caused by its interaction with other proteins (e.g., 14-3-3 proteins).

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SURFACE PLASMON RESONANCE BIOSENSOR FOR DETECTION OF TRAMADOL IN DRINKING WATER

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The quality of drinking water plays an important role in the public health protection. Various diseases, e.g. cholera, are well known to be water-borne. Apart from microbial contamination, chemical contamination with pesticides, pharmaceutical drugs and various other substances may also represent health risks [1]. In order to prevent serious health consequences, the presence of such chemicals in the drinking water needs to be monitored. This requires the development of new analytical methods that are sensitive, selective and affordable and enable in situ analysis.

In this work we present the surface plasmon resonance (SPR) biosensor for the detection of pharmaceutical drug tramadol in drinking water. The developed detection assay utilizes the inhibition format combined with signal enhnancement via functionalized gold nanoparticles. This assay is fast (time of analysis ~ 45 min) and requires minimal sample preparation. The limit of detection (LOD) was determined as 0.36 nM, which is lower than LODs provided by most of the methods that have been developed so far. In order to demonstrate the applicability of the developed assay for the analysis of real samples, tap water from public water distribution network in Prague was also analyzed.

In order to achieve better detection performance of the biosensors, hybrid technologies are pursued worldwide. Currently, we focus on combination of SPR biosensors with electrochemical (EC) methods. We believe that such tandem EC-SPR biosensor will enable detection of various analytes in different matrices with higher sensitivity and selectivity.

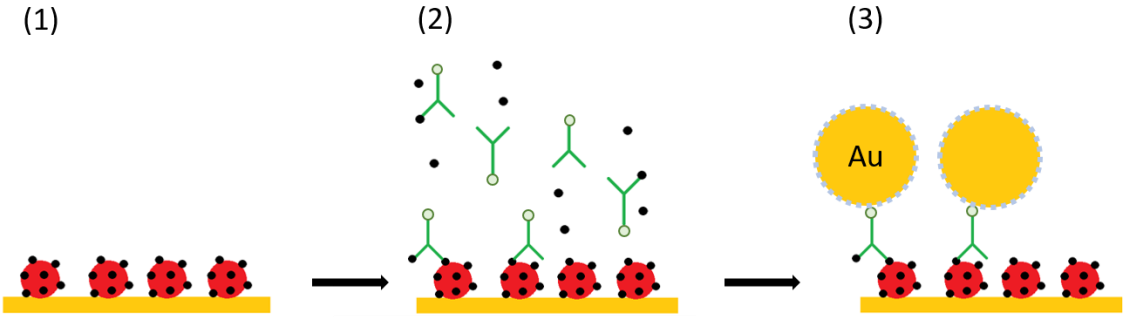

Figure: Schematic representation of the assay for detection of tramadol:
(1) surface of the SPR chip functionalized with the analogue of the tramadol
(2) incubation of the sample with the antibody against tramadol and capture of the unreacted antibody
(3) enhancement of the sensor response via functionalized gold nanoparticles

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THE IMPACT OF THE GLYCAN HEADGROUP ON THE NANOSCOPIC SEGREGATION OF GANGLIOSIDES

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Gangliosides is a term used for diverse group of amphipathic membrane lipids belonging to glycosphingolipids family. Together with sterols (cholesterol primarily) and sphingolipids these molecules show the ability to segregate in membrane forming small, heterogeneous and highly dynamic domains (10-200 nm) referred as 'lipid rafts' by consensus on Keystone Symposium on Lipid Rafts and Cell Function. Their structural hallmark is bulky oligosaccharide headgroup with one or more sialic acids linked to the sugar chain and ceramide lipid tail. The headgroup itself is characterized by high degree of complexity and it draws the attention of many scientists in this field. In this brief study we discuss the role of the headgroup on nanoscopic segregation of gangliosides. In fact, we investigated the effect of the reduction in the number of sugar units of the oligosaccharide chain of three gangliosides: GM_1, GM_2 and GM_3 on biomimetic systems of lipid bilayers called giant unilamellar vesicles (GUVs) applying Forster Resonance Energy Transfer (FRET) - fluorescence microscopy and spectroscopy method.

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BEHAVIOUR OF G-QUADRUPLEX DNA ON ELECTRODE SURFACE MONITORED USING NOVEL ELECTROCHEMICAL PROBES

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The alternative DNA structures known as G-quadruplexes (G4) are of high interest as important regulatory elements, potential cancer therapy targets or building blocks in nanotechnology[1]. In this work, a method for electrochemical study of these structures is presented. Signals of novel electrochemical probes, the porphyrin Cu-TMPy2PP and 3-nitrophenyl modified deoxyguanosine triphosphate ($dG^{NP}TP$), were used for indirect monitoring of the properties of various ODN sequences adsorbed on a hanging mercury drop electrode (HMDE) related to ODN structure in solution. ODNs adsorption on HMDE leads to a significant shift of the characteristic cathodic peak of the probe Cu-TMPy2PP towards more negative potentials compared to the bare HMDE. When HMDE with adsorbed G4-forming ODNs were exposed to highly negative potentials by potential cycling prior to the introduction of the probe, the peak of Cu-TMPy2PP showed gradual transition to its original position as a function of the negative potential value and time of exposition. This observed effect suggested breakdown of the ODN layer which facilitated the probe admittance to bare HMDE surface. This behaviour is in stark contrast to ODNs unable to form G4, where the described effect was not observed. It was ascribed to partial desorption of the adsorbed negatively charged G4-forming ODNs by electrostatic repulsion when exposed to far-negative potentials, creating free electrode surface for direct reduction of the probe. The desorbed ODNs were also detected in the background electrolyte by the highly sensitive radioactive ^{32}P labelling in combination with native polyacrylamide gel electrophoresis. Application of $dG^{NP}TP$ as the electrochemical probe confirmed these results. Furthermore, due to higher sensitivity of the $dG^{NP}TP$ signal, it was shown that ODNs form variably permeable layers on HMDE based on their structure.

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HIGH THROUGHPUT PLATFOR FOR ELECTROCHEMICAL CHARACTERIZATION OF ELECTROGENIC BACTERIA

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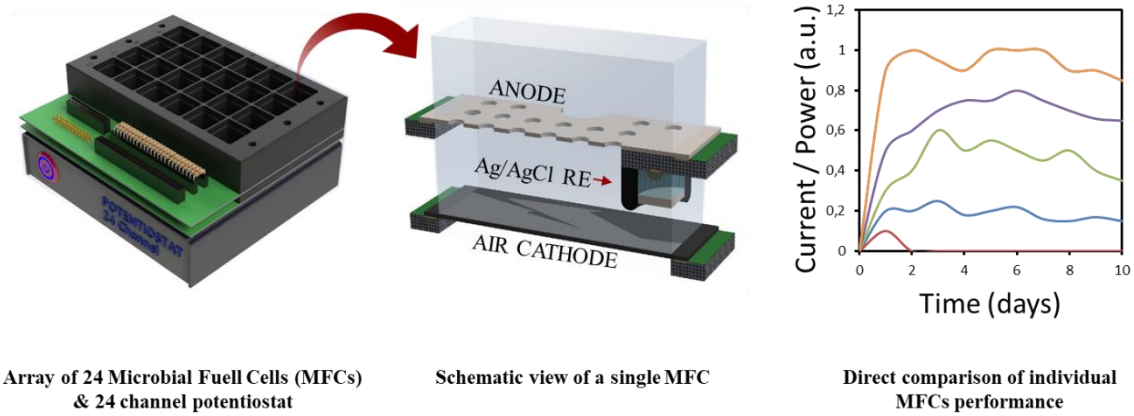
Electrogenic and electron accepting capability of some bacteria strains, are important phenomena which promises advances in the fields of electronically stimulated biotechnological production of valuable chemicals, wastewater treatment, bioremediation, desalination, energy production, novel materials discovery and whole-cell biosensing. Significant boost towards this direction can be achieved with application of high throughput methods known from other biological disciplines. However there is currently a lack of standard and reliable hardware which would enable the same approach in the field of electrogenic bacteria.

Thus we present a platform based on standard Microplate setup with 24 or 96 single chamber air-cathode Microbial Fuel Cells (MFCs) with integrated reference electrode inside each chamber [1,2]. All electrodes are individually addressable by custom multichannel potentiostat capable of performing standard electrochemical measurements including Electrochemical Impedance Spectroscopy. The device enables the direct and parallel comparative analysis of microbes from different sources or under different conditions such as electrode potential, pH and growth medium.



Array of 24 Microbial Fuell Cells (MFCs) & 24 channel potentiostat

Schematic view of a single MFC

Direct comparison of individual MFCs performance

Figure: Schemitic description of the whole concept.

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ANALYZING THE HYDRATION AND MOBILITY OF THE ACTIVE SITE OF HALOALKANE DEHALOGENASE ENZYMES (HLDS)

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Haloalkane dehalogenase enzymes (HLDs) are proteins that cleave the bond in the haloalkane to produce alcohol, halide and hydrogen ion.[1] They are potential biocatalytic agents and biosensors against the toxic haloalkanes. HLDs have an active site where the hydrolysis of the haloalkanes into corresponding alcohols occurs in two steps. First the Aspartate residue in the active site creates the covalent alkyl-enzyme intermediate. In the second step, Histidine residue in the active site activates near water molecules to break the bond between halogen atom and carbon.[2] The substrate reaches the active site through a tunnel whose architecture effects the activity of the HLDs.[3] Thus, it is important to characterize the tunnel properties. For this purpose, fluorescence techniques which enables site specific labelling are chosen. We first mutated the Histidine residue in the active site in the HLDs so that the substrates will not be removed from this active site. Then we used fluorescent substrates 4-Bromomethyl-6,7-dimethoxycoumarin (DMC) and Promega HaloTag® Coumarin to study the mobility and hydration within the tunnel region of different variants of haloalkane dehalogenase enzymes (HLDs). Time Dependent Fluorescence Shift (TDFS), Anisotropy and Quenching methods have been applied to characterize the tunnels.

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CONSTANT CURRENT CHRONOPOTENTIOMETRIC ANALYSIS OF BASIC PROTEINS AT THE CHARGED SURFACES

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Histones are small basic nucleoproteins whose key role is to stabilize negatively charged DNA inside the chromatin. The structural unit of chromatin, nucleosome, consists of DNA double-wrapped around a histone octamer, which is composed of two copies of each core histone, namely H3, H4, H2A and H2B. Core histones contain a large amount of positively charged amino acid residues, such as arginine and lysine [1].

Constant current chronopotentiometric stripping (CPS) analysis is a highly sensitive electrocatalytic method frequently used to study catalytic hydrogen evolution reaction (CHER) of non-conjugated proteins on mercury electrode [2]. As a result of ongoing hydrogen evolution reaction, proteins and peptides yield so-called CPS peak H. In previous work by, Melnikova et al. [3], the CPS analysis was used to determine and analyse the behaviour of five selected proteins, namely human serum albumin, lysozyme, β - synuclein and histones H2A and H3, at charged surfaces. The measured data obtained from both histones significantly differed from the other proteins, which was likely due to presence of high amount of basic amino acids residues, as mentioned above. Based on results of this research, we decided to study the behaviour of the core histones as well as histone octamer adsorbed at negatively charged surface in more detail using label-free electrochemical methods, namely CPS analysis and impedance C_d - t curves. The CPS analysis provides information about histone's involvement in CHER, while the C_d - t curves reflect changes of the electrical double layer on a charged electrode surfaces [1].

Based on the information obtained from both electroanalytical methods, our studies show that CPS responses of histones and their subsequent involvement in CHER is influenced not only by the basic amino acid residues content but also by an accessibility of free C- and N- terminal tails to the electrode surface. Additionally, we confirmed that with CPS analysis, it is possible to determine whether the histone octamer is a complex structure and not only a mixture of core histones.

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ELECTROCHEMICAL BIOASSAY COUPLED TO LAMP REACTION FOR DETERMINATION OF HIGH-RISK HPV INFECTION IN CRUDE LYSATES

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Cancer biomarkers play a pivotally important role in early-stage cancer diagnostics. DNA biomarkers include for instance DNA mutations, abnormally methylated DNA, DNA from viruses that are associated with cancer, or circulating tumor DNA. DNA electrochemistry technique can be a powerful analytical technique for investigation of cancer biomarkers because it (a) requires small, low-priced instrumentation that is easy to operate, (b) is simple, (c) can be miniaturized and (d) is adjustable for high-throughput screening [1, 2].

Similarly to optical detection techniques, detection of DNA biomarkers by electrochemistry mostly requires DNA extraction step to remove other cellular components, which is laborious and time-consuming. Using LAMP (loop-mediated isothermal amplification) technique is one option to circumvent this step. LAMP is an isothermal amplification technique that can amplify DNA directly in crude lysates in a short time (30-40 min) at a constant temperature (usually between 60 °C and 70°C) [3]. Here, we showed that human papillomavirus (HPV) can be sensitively detected by a novel LAMP-based EC method directly from the crude lysate of cancer cells. We have chosen HPV16 and HPV18 as the two most frequent HPV types that are a major etiological factor of cervical cancer in women[4].

To validate our method, we tested not only cancer cell lines but also clinical samples ufrom the gynecologist ranging from healthy women to women suffering from cervical precancerous lesions caused by HPV 16 or HPV 18 genotypes. We reached excellent concordance of our assay with PCR, obtaining 100% sensitivity for both genotypes, >80% specificity for HPV 16 and >94% specificity for HPV 18. To sum up, we showed here for the first time an electrochemical platform that is capable to detect DNA from highly oncogenic HPV directly in crude lysates.

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BORONIC ACIDS AND FERROCENES – FROM ELECTROACTIVE MOLECULAR PROBES TO ROS-ACTIVATED PRODRUGS

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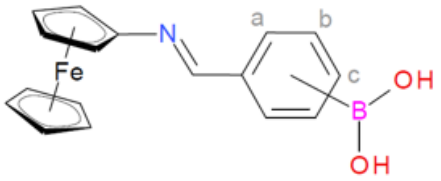
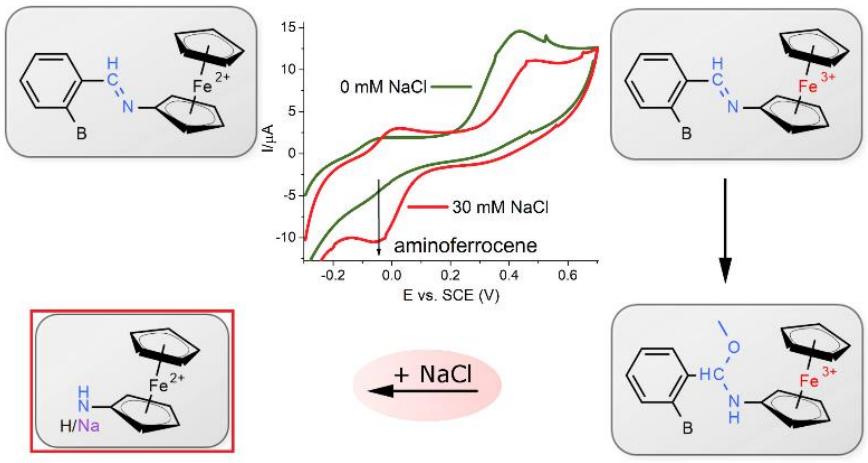
A chemosensor is a device containing a recognition, a detection, and a transducer unit. It contains small chemical molecules, i.e., molecular probes as signalling elements. The field of (chemo)sensors is already well-developed, especially its part with an optical detection.[1] However, probes possessing an electrochemical detection are still quite scarce.[2]



Hence, our research activities in the field of synthetic and material chemistry started with synthesis, structure elucidation and description of physicochemical properties of derivatives including boronic acids and ferrocenes, especially new aminoferrocene probes.[3] Because an *ortho*-arylboronate has been often employed in enhancing of diol binding[4] which is then effective even at physiological neutral pH, we synthesized and compared three different ((ferrocenylimino)methyl)phenylboronic acids (a, b, c) and their catechol esters, respectively. The further work involved description of their behaviour in electrochemical processes determining functional abilities of the synthesized derivatives using voltammetric techniques and spectroelectrochemistry.



The *ortho*-iminoboronate isomer appeared to be an excellent structure for studies of the donor–acceptor properties, (proton-coupled) intramo-lecular electron transfers and intramolecular inter-actions in general. These involve the atomic B–N and B–diol interactions in Lewis's definition. Fur-thermore, we described electrochemically facilitated reactions on an electron-rich imine bond (redox-controlled, i.e., redox-dependent addition of *O*-nucleophiles).[5] As a consequence, we also observed complete

redox-dependent breakage of imine bond in non-aqueous solvent in the presence of NaCl facilitated by electrochemical cycling of the *ortho*-iminoboronate.[6]

Current possibilities of utilization of electroactive boronic acids lay not only in a molecular recognition (analytical sensing) but also in drug development (drug delivery and on-demand release). Such a functional material arisen from this research field can be applied as an enzyme inhibitor or an advanced boronic acid-based smart material with properties of stimuli-responsiveness. The reactive oxygen species (ROS), pH or diols can serve as a stimulus for a coupled functional task. At last, but not least, also ferroptosis and biochemical processes leading to e.g., anticancer activity which deal with elevated or suppressed levels of ROS and pH in cells, can be influenced by these electroactive boronic acids as (pro)drugs.[7] First trials examining cytotoxic abilities of the iminoboronates have been currently done in which *para*-iminoboronate catechol ester showed slightly higher cytotoxicity than aminoferrocene as a control.[8]

ACKNOWLEDGEMENT

This research has been financially supported by the Ministry of Education, Youth and Sports of the Czech Republic under the project CEITEC 2020 (LQ1601), by Masaryk University under the project (MUNI/A/1424/2019), and by the Czech Science Foundation, grant nr. 19-16273Y. We also acknowledge CF Nanobiotechnology supported by MEYS CR (LM2015043) and CEITEC Nano+ project CZ.02.1.01/0.0/0.0/ 16_013/0001728.

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BIOPHYSICAL CHARACTERISATION OF PHOSPHORYLATED 14-3-3ζ PROTEIN

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Phosphorylation is one of the main post-translational modifications of proteins that govern biological systems. 14-3-3 proteins are regulatory protein hubs that are engaged in cell cycle and apoptosis, modulation of enzymatic activities, cytoskeleton or signalling pathways [1]. They normally exist as dimers [2]. It has been proposed that phosphorylation of a single Ser residue (Ser58) at the dimeric interface, induces monomerization of 14-3-3 protein [3,4]. However, the problematic preparation of specifically and fully phosphorylated 14-3-3 protein (pS58) has impeded exploration of the actual effects of phosphorylation for a long time. Instead, most studies have used monomeric or phosphomimicking mutants to approximate properties of the 14-3-3 pS58 protein [5,6,7].

Here, we present the characterisation of 14-3-3ζ pS58 protein employing a wide range of biophysical techniques. Methods, such as electrophoresis, analytical ultracentrifugation, UV-Vis spectroscopy, light scattering, circular dichroism, fluorescence techniques, nuclear magnetic resonance, calorimetry or surface plasmon resonance, were used to obtain insight into the oligomeric state, structure, thermal stability, hydrophobicity and binding abilities of the phosphorylated protein. All results were compared with the non-phosphorylated protein (wild type), monomeric and phosphomimicking mutants.

We have found that phosphorylation of Ser58 effectively shifts the dimer-monomer equilibrium to the side of monomers, decreases thermal stability and increases hydrophobicity of the protein. Moreover, it significantly affects the interaction between 14-3-3ζ and Tau protein, which may be relevant for Alzheimer's disease pathology. Finally, it was revealed that the phosphomimicking mutant behaves differently and therefore should not be considered as an adequate replacement of phosphorylated 14-3-3ζ protein.

ACKNOWLEDGEMENT

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ELECTROCHEMISTRY OF POLYACYLGERMANES, -SILANES, AND -STANNANES

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Acylgermanes are challenging, innovative class of compounds with attractive application possibilities in photochemically induced polymerizations. Moreover, due to their low toxicity, they are frequently used in human medicine for white dental fillings (Ivocerin®, the corresponding photo-induced reaction mechanisms are well established [1]).

Upon visible light irradiation, C-Ge bond is cleaved and radicals are formed. The absorption spectra of the photoinitiators depend on the electron donating/withdrawing character of substituents at the aromatic moieties [2-3]. Push-pull effects, however, also indicate a substantial effect on the redox properties of the acylgermanes monitored by means of electrochemical methods. The first electron reduction of the parent compounds yields an anion radical.

In this context, we present our results obtained by DC-polarography and cyclic voltammetry across a series of mono-, di-, tri- and tetraacylgermanes, di- and tri-nuclear polyacylgermanes, analogical silanes and stannanes as well as other related compounds [4]. The observed trends in their first reduction potentials will be discussed in terms of the substitution patterns at the central atom.

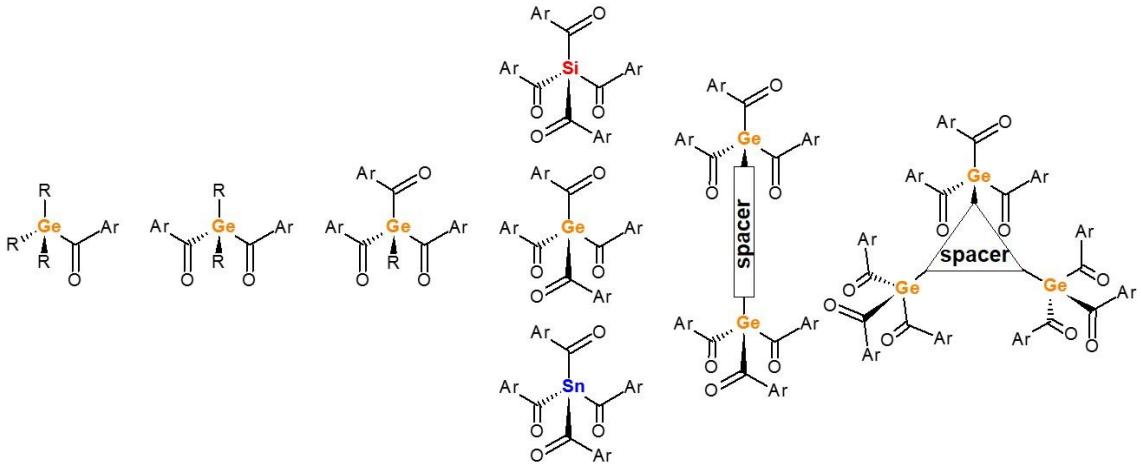

Figure: Structures of investigated compound classes (Ar=aryl, R=alkyl).

ACKNOWLEDGEMENT

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SPECTROSCOPIC BINDING STUDIES OF NEUROBLASTOMA DISEASE MARKERS BY NOVEL THIOPHENE BASED RECEPTROS-PRECURSORS FOR THE ELECTROCHEMICAL SENSORS' PREPARATION

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The key role of anionic species in biological and environmental issues is well recognized and the problems associated with disrupted balance of anion in the nature represent currently a hot topic. Besides that, multitude of the negatively charged species are also known as precursors of various illnesses and the monitoring and sensing of these structures could be useful in diagnostic [1]. Nowadays one group of promising and successful material are electrochemical sensors. These sensors using conducting polymers such as polypyrrole (PPy), polythiophene (PT) etc. are low-cost, portable and successful instruments generally adopted in clinical analysis [2].

Therefore, the aim of our research is preparation of anionic receptors utilizing the thiophene skeleton as polymerizable carrier. New receptors were designed and synthetized according to required binding features. The anionic metabolites, known as tumour markers of neuroblastoma such as homovanillic acid (HVA) and vanillylmandelic acid (VMA) were screened by the monomeric thiophene-based receptors with urea binding site. Complexation properties and association constants were then evaluated using the NMR and UV-Vis titration experiments. Finally, receptor selectivity to HVA and VMA among other carboxylates was assessed.

ACKNOWLEDGEMENT

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BIOLUMINESCENT PROBE FOR CASPASE IMAGING INSIDE CELLS BASED ON FÖRSTER RESONANCE ENERGY TRANSFER BETWEEN QUANTUM DOT AND QUENCHER

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Nowadays, luminescent semiconductor quantum dots (QDs) are widely applied in different areas due to their unique optical properties. As an example, QDs are used as photoluminescent labels with excellent possibilities for high-throughput detection and diagnostics. Modern technologies and instrumentations of laser-induced fluorescence or bioluminescence offer the possibility to study biological phenomena at a cellular or even molecular level. Progress in bioanalytical techniques has accelerated the deep understanding of cellular processes [1,2].

Our research is focused on biologically active molecules, such as caspases, which play important roles in cell signaling regulation in normal and diseased states and are attractive targets for biological diagnosis and also for medical therapy [2-6]. To analyze the biological events in single cells, technologies related to fluorescence and luminescence imaging advanced rapidly in the past two decades. Three prominent types of fluorescent molecules have been used for bioimaging: fluorescent proteins, artificially synthesized organic dyes, and fluorescent nanoparticles including QDs [1, 7-11]. Compared with traditional luminescent organic dyes, QDs exhibit excellent photophysical properties, a high photostability, broad excitation and narrow symmetric emission bands. The emission wavelengths of QDs, dependent on their sizes, are tunable by particle synthesis. Moreover, their high extinction coefficients make them ideal for absorption and transfer of relatively large amounts of energy [12-14]. In addition, QDs are suitable as Förster resonance energy transfer (FRET) donors for optical sensors [15, 16]. According to the Förster definition [17], FRET is a photophysical process by which the energy of the donor luminophore in excited state is nonradiatively transferred to the acceptor luminophore, and then emitted as a longer wavelength photon. The immediate nonradiative process is based on dipole–dipole interactions between a donor and acceptor in the range of 1–10 nm [18, 19].

The implementation of QD as FRET donors brings several advantages [20, 21]. High brightness, quantum yields, and long photoluminescence times are prerequisites of extraordinary energy transfer rate. Similarly, the energy transfer rate is increased by the attachment of multiple acceptors to the large surface area of QDs. The size tunability of luminescence spectra is advantageous for the optimization of spectral overlap between donor and acceptor. Moreover, the broad absorption bands allow an effective excitation of QDs at wavelengths low enough not to directly excite acceptors.

In this work, synthesis and testing of a novel quantum dot luminescent probe is presented. The probe was synthesized in two steps. In the first step, water-soluble QDs were conjugated with a specifically designed peptide using a ligand-exchange approach, where the SH- group of cysteine at the end of the peptide substitutes the mercaptosuccinic acid at the QD surface by forming covalent bond with the Cd atom in nanoparticle crystal lattice. In the second step, the opposite terminal amino group of the peptide reacts with the BHQ-2 quencher via succinimidyl group. The synthesized luminescent probe was tested by a model reaction with active human recombinant caspase-3 protein in quartz cuvette of a fluorimeter. The caspase enzyme reaction is based on the specific cleavage of the DEVD peptide sequence. Thus, the BHQ-2 quencher is released, the Förster resonance transfer between quantum dot and quencher is interrupted, and consequently the red light luminescence of the quantum dot is emitted (**Figure 1**). Luminescence emission spectra of the reaction were recorded in time in the range from 550 to 650 nm with excitation at 405 nm. Thus, due to the high stability, the synthetized QD FRET-based luminescent probes proved to enable much longer observation of active caspases in living cells under fluorescence microscope than commercially available probes.

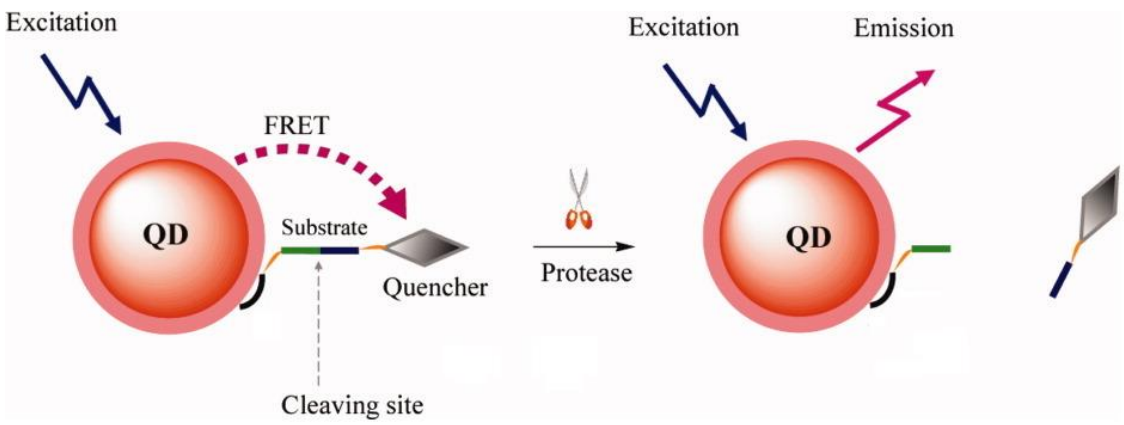

Figure 1: Scheme of the quantum dot FRET-based luminescent probe reaction with cleaving enzyme.

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NITRO GROUP AS BINDING/RELEASE SWITCH IN UREA-BASED RECEPTORS

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Anions play an important role in biological systems. At the same time, many of them are the major environmental pollutants and substances endangering living organisms. For these purposes, anion recognition and elimination would find application in many branches. Among the most widespread anionic pollutants are phosphates, which are known to cause eutrophication of water. Moreover, their limited removal from body fluids can lead to serious health issues.[1] Inspired by nature, supramolecular chemists have synthesized a variety of compounds with remarkable selectivity and efficiency for particular anionic species.[1] Despite numerous studies dealing with the preparation of receptors with suitable binding affinity to a targeted anion, systems offering the controlled possibility of subsequent anion release are rather rare. Since the complexation behavior of a receptor is influenced by the electron density surrounding the binding site, its electrochemically induced change can be used as a binding/release switch. On condition that the receptor contains an electrochemically sensitive group as an integral part of the structure, the binding affinity of the receptor depends on the redox state of such a probe, i.e. in general, the oxidized form of receptor enhances anion binding and *vice versa*, a reduced form of the receptor leads to a drop of anion binding efficiency.[2] Therefore, the aim of our research is the design and synthesis of anionic receptors, with suitable binding sites for phosphates, as well as the determination of their binding ability and electrochemical behavior. In our work, redox-sensitive receptors contain a conjugated system of urea-based binding site with electrochemically switchable nitro group. The change between electron-withdrawing effect of the nitro group to the electron-donating effect of an amino group was studied for receptors bearing the probe group in *o*-, *m*- and *p*- positions.

Table: Association constants K_{Ass} measured by UV-Vis titration for series of receptors with $TBA^+H_2PO_4^-$ in DMSO and corresponding BEFs.

Position of substitution	$-NO_2$	$-NH_2$	BEF
para	33 000	1 100	30
meta	15 800	2 800	5.6
ortho	2 400	6 300	0.4

BEF – Binding Enhancement Factor. Error, when estimated, was < 5 %

According to the obtained results the *para*- substituted derivative was chosen for further study in different polar aprotic solvents for obvious reasons. Apart from the known affinity of the urea-based receptors towards DMSO itself, the switching of binding efficiency must also be possible in solvents used for electrochemical experiments. In the case of ACN the redox pair showed up to an 80-fold decrease in anion binding affinity upon nitro group reduction. So the principle of redox switchable receptors was justified by the thorough study. Moreover, detailed mechanisms of possible electrochemical reductions were studied. The CV and spectroelectrochemical experiments revealed that the complexation of dihydrogen phosphate completely changes the reduction mechanism. The urea complexation site occupied by hydrogen phosphate does not deprotonate and changes the auto-protonation mechanism to a standard two-step reduction process.[3] Although the used conditions did not allow the full reduction of the nitro group to amine, the substituent effect of hydroxylamine is sufficient to allow the application of the nitro group incorporated into the structure of newly designed receptors as a redox affinity switch for the anion complexation/release.

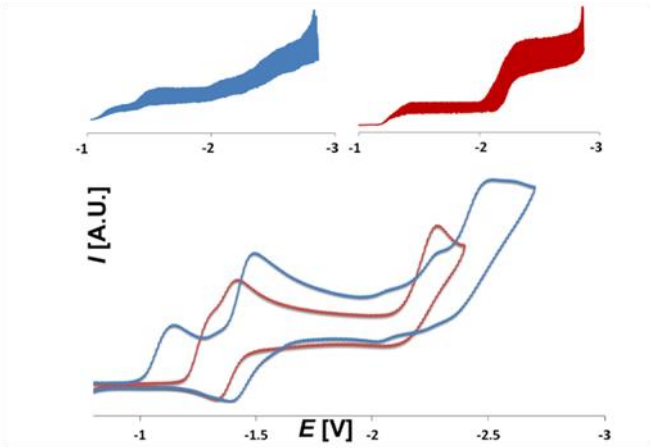

Figure: Polarographic curves and cyclic voltammograms illustrating the reduction of *para*-NO_2 receptor (blue) and *para*-NO_2 receptor in the presence of 12 equivalents of $TBA^+H_2PO_4^-$ (red) in DMF.

ACKNOWLEDGEMENT

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PHOTON-UPCONVERSION NANOPARTICLES FOR THE DETECTION OF HONEYBEE PATHOGEN *PAENIBACILLUS LARVAE*

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The western honeybee (*Apis mellifera)* is the most frequent pollinator of flowering plants, making it exceptionally important for agriculture and biodiversity. However, bees are being threatened by various diseases, including bacterial infections. The bacterium *Paenibacillus larvae* is the causative agent of American foulbrood (AFB), the most destructive disease of the honeybee brood. The treatment can be carried out using the antibiotic oxytetracycline (OTC). However, if used, residues can be found in honey and other bee products, which lowers their quality. This is the reason why OTC is banned in many countries in Europe, for example, the Czech and Slovak Republics. In such countries, the only means of the disease management is to burn whole colonies, making rapid and sensitive detection necessary to limit the spreading of this pathogen.

A traditional method for *P. larvae* detection is polymerase chain reaction (PCR), which allows for very sensitive analysis with high sample throughput. However, the use of this method is often limited by contaminations, as well as PCR-inhibitors present in the bee material. This then makes the DNA isolation and purification a possible source of complications. An adequate alternative to the conventional diagnostic methods are antibody-based techniques, such as enzyme-linked immunosorbent assay (ELISA). These assays rely on the signal generated by enzymes, typically horseradish peroxidase. ELISAs are considered the gold standard of immunochemical methods for their high sensitivity, specificity even in complex matrices, and the ability to detect a wide range of analytes. Nevertheless, the conventional ELISA is not sensitive enough for the detection of early stages of the AFB.

The recent progress in nanotechnology has provided various nanomaterials that can be used as sensitive labels in immunoassays to enhance their sensitivity. Particularly, photon-upconversion nanoparticles (UCNP) are promising alternative luminescent labels in immunoassays. They are unique for their anti-Stokes luminescence that can be excited by the NIR laser and detected in the Vis region with no optical background interference. After the surface modification step, UCNPs can carry antibodies or streptavidin on their surface and thus be used as labels in various immunoassay formats, including the microtiter plate-based upconversion-linked immunosorbent assay (ULISA) [1].

We have developed a sandwich ULISA for the diagnosis of AFB, using UCNPs conjugated with antibody or streptavidin, and compared the results to the conventional ELISA. Specific polyclonal antibodies were prepared *via* immunization of New Zealand white rabbits and tested in a standard sandwich ELISA, which achieved an LOD of 6.5×10^4 CFU/mL. The UCNPs were modified using an Alkyne-PEG-neridronate linker, which allowed their bioconjugation with

azide-modified antibody or streptavidin using copper-catalyzed click chemistry. The alkyne on one side of the linker was connected to the azide on the Ab or SA in a click-reaction, whereas the neridronate on the other side strongly coordinated to the UCNP surface. Firstly, the UCNPs were characterized using transmission electron microscopy (TEM) and dynamic light scattering (DLS). Results from the TEM analysis have shown an average UCNP size of 58.5 nm. DLS was used to define the hydrodynamic diameter, which was 54.2 ± 1.7 nm before and 88 ± 2 nm after the conjugation with antibody; the streptavidin conjugate has reached the average size of 97 ± 3 nm. The synthesized labels were then employed in a sandwich ULISA, in which the bacteria *Melissococcus plutonius*, *Paenibacillus alvei*, and *Brevibacillus laterosporus* were used as negative controls (**Figure 1**). These bacteria are relevant as they also invade the honeybee brood. The results showed low cross-reactivity with *Paenibacillus alvei* and no cross-reactivity with the other bacteria.

The antibody-based UCNP conjugates have shown specific binding; however, the achieved LOD of 4.7×10^6 CFU/mL was slightly worse than in the case of ELISA. This may be due to the antibody being of polyclonal nature. When the polyclonal antibody contains a high percentage of IgG molecules not specific for the analyte, some of the nanoparticles might not show a specific binding due to the limited number of alkyne groups on the surface. On the other hand, the streptavidin-based labels achieved the LOD of 2.9×10^3 CFU/mL, which represents a 22-fold improvement in comparison with the ELISA. Finally, the potential for practical use of the ULISA was successfully demonstrated by the analysis of real samples of spiked larvae, adult bees, and bottom hive debris [2].

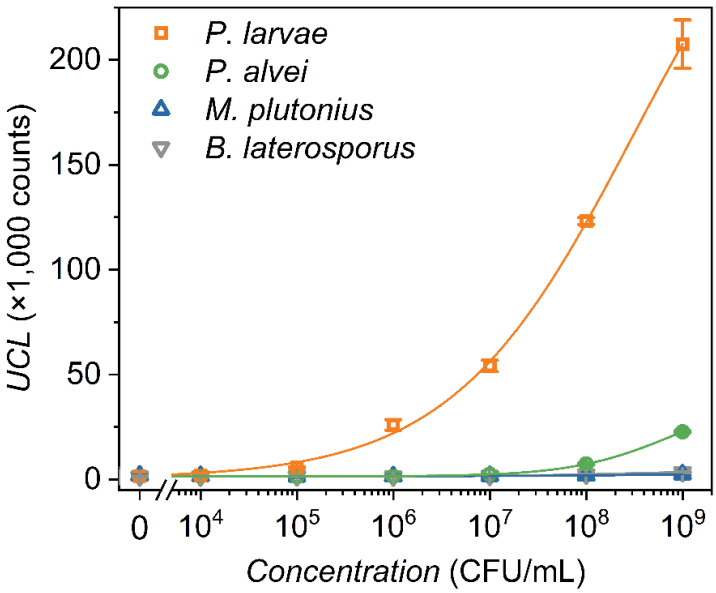

Figure: Sandwich ULISA for the detection of *P. larvae* with *P. alvei*, *M. plutonius*, and *B. laterosporus* as negative controls.

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PROBING STRUCTURE OF LIQUIDS WITH PHOTOELECTRON SPECTROSCOPY

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A molecular and supramolecular structure of liquids is difficult-to-obtain information since liquids stand in the middle of gases and solid-state phase, where methods like molecular spectroscopy on the one side and X-ray diffraction on the other side are commonly available. Photoelectron spectroscopy is a method based on ejecting an electron from a molecule using electromagnetic radiation and measuring the kinetic energy of the electron. It has been commonly used for molecules in the gas phase and surfaces for decades. [1] More recently, a technique of liquid-jet photoelectron spectroscopy enabled measuring data also for liquids. [2] The electronic structure of molecules revealed by this method reflects in a nontrivial way the supramolecular structure of the system. We demonstrate on the example of two molecules – indole and glucose – that this knowledge is often only available when combining experimental data with theoretical calculations.

Indole is a ubiquitous component of peptides and proteins as the essential amino acid tryptophan is an indole derivative. It is suggested to play an important role in protecting organisms from radiation damage through a cascade of inter- and intramolecular redox processes. [3] Recently, a full photoelectron spectrum (valence, carbon core, and nitrogen core ionizations) of indole in water was measured by the laboratories of Dr. Bernd Winter and Prof. Jochen Küpper. Modeling spectra theoretically is a challenging task due to the need for properly describing solvating effects on the energetics of studied processes. Our calculations revealed an interplay between specific and nonspecific solvent effects resulting in a significantly different solvent shift (i.e. a difference between solution-phase and gas-phase ionization energy) for different types of ionizations. This calculated phenomenon agrees with the experimental data and adds further insight into the structure of the closest solvent molecules to the indole. [4]

Glucose is the most famous monosaccharide serving as the energy source for living organisms as well as a building block of many oligo- and polysaccharides. Being a polyprotic acid with 5 hydroxyl (–OH) groups, there are 5 possible sites of single deprotonation. A direct experimental site-selective probe of this deprotonation in an aqueous solution is missing. We theoretically studied possible changes in photoelectron spectra upon deprotonation, i.e. for neutral and basic solutions. We found differences in the spectral shapes in agreement with the experimental data measured by the laboratory of Dr. Bernd Winter. While valence ionizations were insensitive to the deprotonation site due to the delocalization of valence orbitals on the glucose molecule, we revealed substantially different features in the case of ionization of low-lying core electrons. Our calculations demonstrate that there is only one deprotonation site that matches with the experiment (Figure 1), therefore clearly revealing the site-selective information. [5]

The concept of combining experimental data and theoretical calculations in photoelectron spectroscopy of liquids provides valuable information on the molecular structure of solutes as well as solvating molecules.

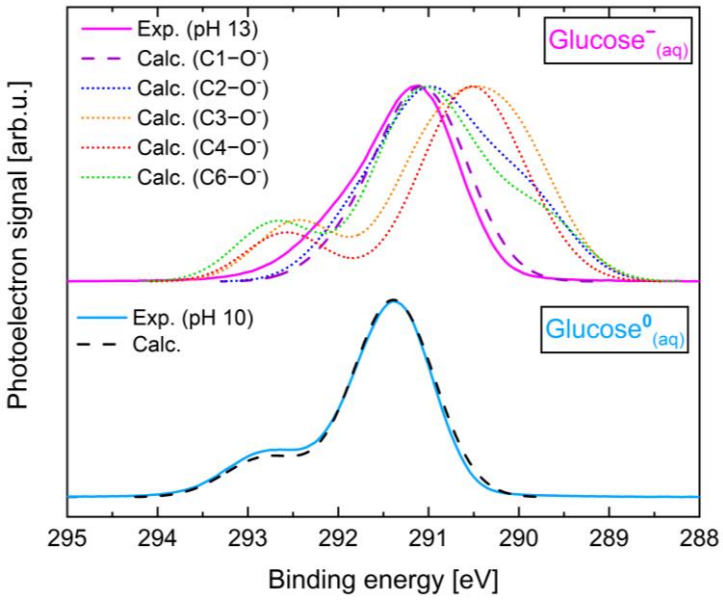

Figure: Experimental and calculated photoelectron spectra of the protonated (bottom) and deprotonated (top) glucose molecule. Calculations reveal only one possible deprotonation site that matches the experiment.

ACKNOWLEDGEMENT

We thank the collaborating laboratories of Dr. Bernd Winter and Prof. Jochen Küpper, who provided the presented experimental data. Support by the Czech Science Foundation, project no. 21-26601X (EXPRO), Specific University Research grant no. A2_FCHI_2021_028, and the OP RDE registration no.: CZ.02.2.69/0.0/0.0/19_073/0016928, funded by the ESF is acknowledged.

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POSTERS

APPLICATION OF TRANSESTERIFICATION DERIVATIZATION REACTION FOR GC-MS ANALYSIS

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Derivatization is a common technique used in chromatography analyses which utilizes the change of function group (such as hydroxyl, carboxyl, or thiol) of the compound. In GC-MS analysis, we encounter problems like low volatility, thermal stability, and poor separation of compounds while using derivatization, it is possible to increase these properties. The derivatization techniques (*e.g.*, silylation, acylation, alkylation, and esterification/ transesterification) are mainly employed for GC-MS analysis [1, 2]. Transesterification is process in which ester (in our work oil) is allowed to chemically react with alcohol. In this reaction, methanol and ethanol are commonly the most used alcohols because of their low cost and good availability.

Lipids are esters of higher carboxylic acids. More specifically, they are fatty acid derivatives of a monohydric or trihydric alcohol. Simple lipids are divided into fats, oils, and waxes. The main components of oils are triacylglycerols - where glycerol is esterified with three fatty acids [3].

In this work, we analysed rapeseed oil (made in the EU, manufactured by PALMA company, Slovakia) by GC-MS. The samples have been derivatized by transesterification using methanol with a basic catalyst (KOH/NaOH as a methanolic solutions of different concentrations).

The oil sample was dissolved in isooctane, and then the solution of derivatization agent was added. The mixture was shaken for defined time, then the solution was let until the moment when two phases have been separated. The upper layer (isooctane with derivatized oil) was transferred to the small vial for GC analysis and then injected on capillary column DB – 5ms 60 m × 0.25 mm × 0.25 μm with Rxi Guard column 1 m × 0.53 mm for separation. Analysis was performed on Agilent HP 6890 Series with mass spectrometer as detector. The highest signal (intensity) from the MS detector for methyl esters was used for the optimization of derivatization process leading to the highest sensitivity, and the lowest LOD and LOQ under those experimental conditions. The results are presented in Figure 1.

The best concentration of hydroxide methanolic solution for transesterification of oil was found (KOH - 3.0 mol dm^{-3}, NaOH 2.0 mol dm^{-3}). At the higher concentrations of hydroxide solution, the side chemical reaction - saponification predominates over the transesterification, causing a lowering yield of methyl esters (ME) of fatty acids which results in low separation between non-polar and polar phases, increased viscosity of solution of derivatized samples as well as lower intensity of MS analytical signals.

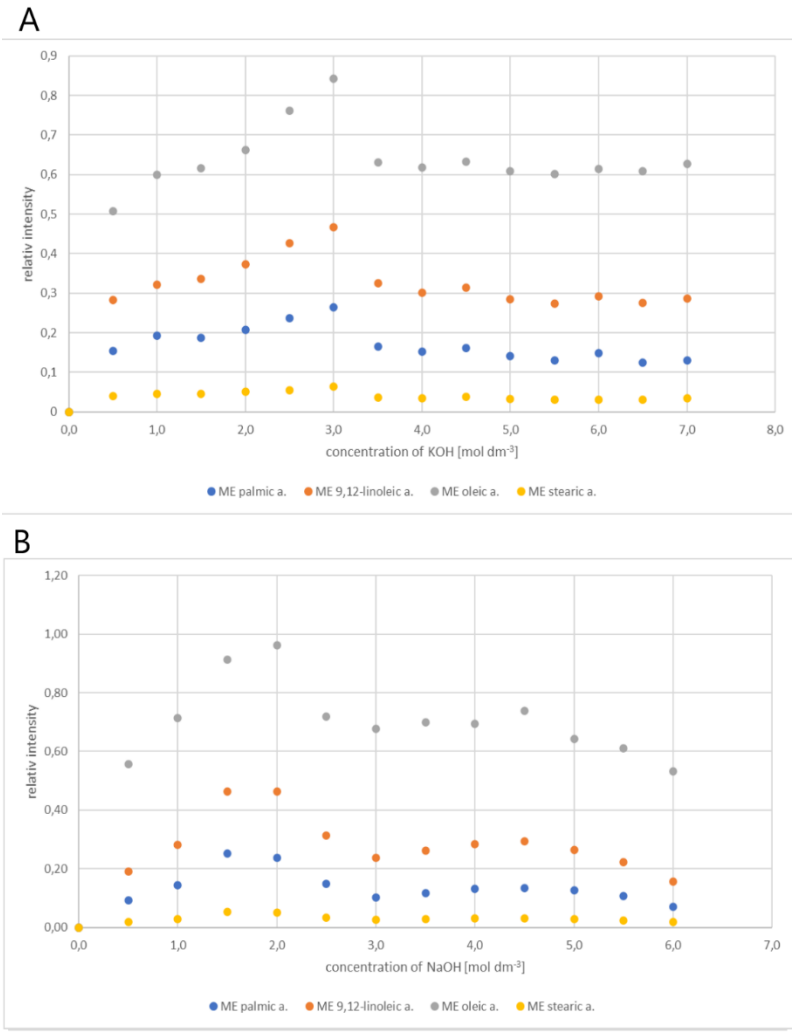

Figure: A – dependence of relative intensity of methylesters (ME) on concentration of potassium hydroxide; B – dependence of relative intensity of methylesters on the concentration of sodium hydroxide.

ACKNOWLEDGEMENT

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AUTOMATION OF EXPERIMENTAL DATA PROCESSING

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Working in an analytical laboratory brings large amounts of data that need to be subsequently evaluated. Applications prepared using programming languages can be used for the evaluation, which can automate this process and simplify data manipulation. They also bring more efficient, as well as faster and more accurate data processing.

In this work, programming languages Python and R were used to create applications for this use. Python and R were chosen because they are free, explicit, productive, and easy to use.

Python is a programming language for general purposes. Using libraries, it is possible to easily create applications with many features. For example, library PyQt5/PySide2 is used for constructing GUI, matplolib for drawing graphs, NumPy and pandas for efficient data processing.

R is a programming language and environment for statistical computing and graphics. Its potential to view objects and interact with them can be enhanced using integrated development environment RStudio. R Markdown is an RStudio-included package which compiles R Markdown files into different formats, such as PDF, HTML, or DOCX.

In Python, we created an application *Titral*. *Titral* is intended for determination of the equivalence point from the titration curve by the derivative method. User imports obtained experimental data and the application returns first and second derivation, draws charts (titration curve and derivations), and shows calculated equivalence point. The window of prepared application is shown in Figure 1A.

Using RStudio, a script that generates a complete protocol for one task from analytical chemistry practicals was created. After importing measured data from MS Excel spreadsheet, the application identifies and rejects outliers in calibration data using Grubb's T-test, constructs a graph using a linear regression model, and prints the regression line equation. Dixon's Q-test is used for outliers detection in the unknown sample data. Furthermore, the application calculates content of analyte in the unknown sample, including standard deviation and confidence interval. LOD and LOQ are also determined. The output of the R script is a clear PDF protocol containing tables with all the measured and calculated data, graphs, used equations and calculations. A portion of this protocol is shown in Figure 1B.

Both prepared applications for data processing work efficiently and are easy to use. They show that programming languages Python and R are suitable for developing programs that make the process of evaluation of data measured in the analytical laboratory faster, more effective, and easier. Saved time enables to measure more data.

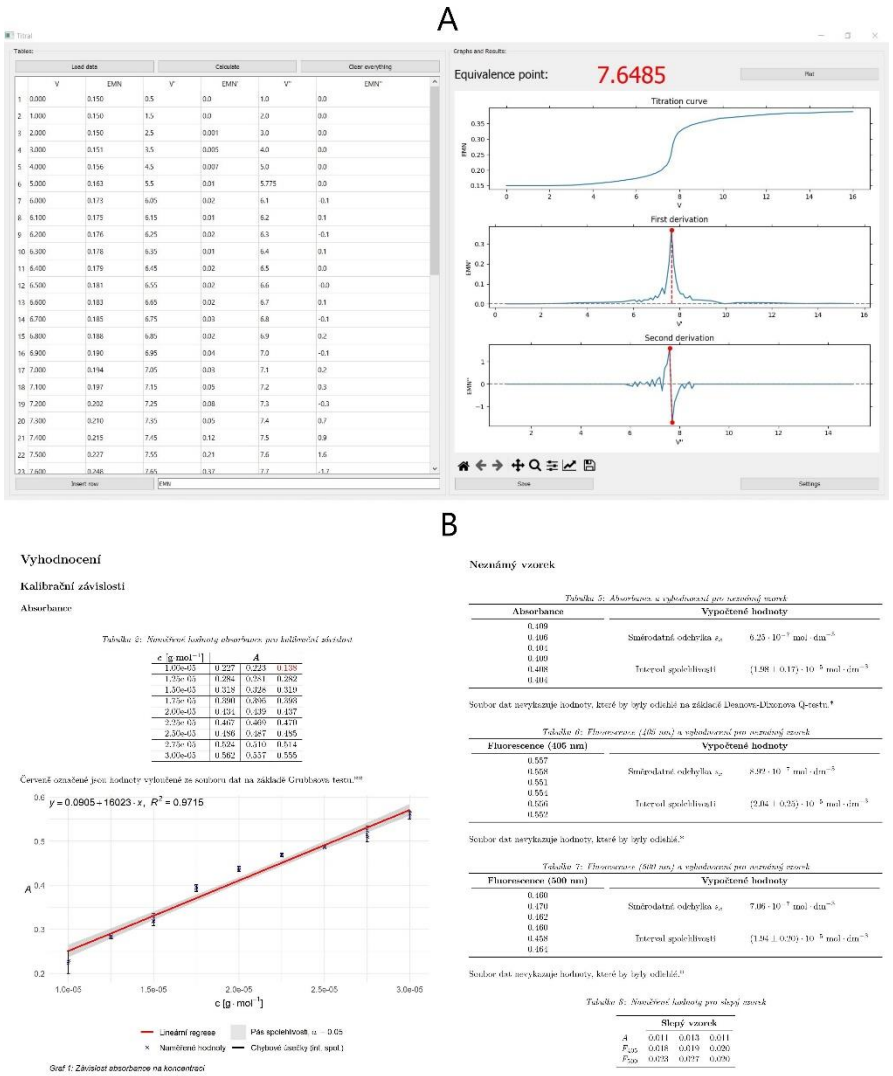

Figure: A – example of window of application *Titral*; B – example of output from R

ACKNOWLEDGEMENT

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ISOTACHOPHORETIC ANALYSIS OF TEA

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Tea is one of the most popular and consumed beverages in the world because it is a cultural drink with a long history prepared by infusing tea leaves in hot water. The tea tree is a noble evergreen plant of genus Camellia, grown in subtropical and tropical areas [1,2].

Tea contains many different substances that affect its taste, aroma, and colour. Volatile fragrant substances, which make up only 0.01% of the weight of the tea leaves, are primarily responsible for the taste and aroma. Polyphenols (*e.g.*, flavonoids, epicatechin, epicatechin-3 gallate, epigallocatechin-3–gallate, epigallocatechin, *etc.*) are present in large quantities (up to 30% w/w). The most important component of tea is caffeine, sometimes called theine, found in tea leaves in varying amounts (3–7% w/w). The tea leaves also contain Ca(II), Mg(II), Na(I), Fe(II)/Fe(III), chloride, fluoride and phosphate ions, as well as vitamins C, E, B1, and B2. To a lesser extent are present sugars, waxes, and fibre [1]. Appreciable influence on the taste and quality of tea does have also inorganic minerals sulphate and phosphate and anions of organic acids (*e.g.*, tartrate, malate, acetate, *etc.*).

Isotachophoresis (ITP) is an electromigration technique used to analyse and isolate ionic compounds. It is performed in a discontinuous electrolytic system consisting of a leading (LE) and a terminating (TE) electrolyte. The leading electrolyte should have a higher and the terminating electrolyte lower mobility than analysed ions present in the sample. The sample is injected between these two electrolytes, however, at low concentrations of analytes, the sample can be injected into the leading or terminating electrolyte. After applying voltage, the analytes are divided into zones according to their mobilities. When equilibrium is reached, all zones migrate by the same velocity while only cations or anions can be separated during one ITP run. [3,4].

In this work, the ITP was applied for the analysis of loose black teas and teabags. The analytes of interest were sulphate, tartrate, malate, acetate, and phosphate ions. The ITP analyses were carried out on electrophoretic analyser EA 102 (Villa Labeco, Slovakia) with a contact conductivity detector. The column length was 180 mm, and the diameter of the PTFE capillary was 0.3 mm. The analyses were performed in a two-step mode with 70 μA/30 μA current. The LE composition was 10 mM HCl + 5.5 mM BIS-TRIS propane, pH = 6.6; the TE was 10 mM hexanoic acid (pH = 7.2). In Figure 1, one can see example of ITP record for selected tea samples. All anions of interest were detected and their concentrations estimated in analysed tea samples. In almost all teas, it was found the highest amount of malate.

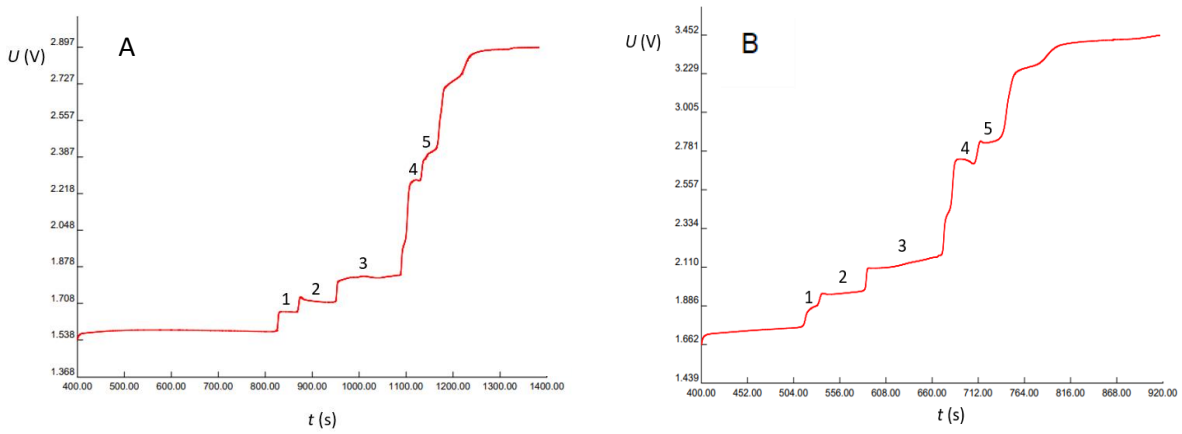

Figure: ITP records of selected tea samples:
A – Oxalis Mangalam BPS CL (loose tea), B – Jemča – black tea (teabag)
1 – sulphate, 2 – tartrate, 3 – malate, 4 – acetate, 5 – phosphate

ACKNOWLEDGEMENT

The work has been supported by Masaryk University (MUNI/A/1192/2020) and Ministry of Education of the Czech Republic (LTC20044).

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HYDROGEN EVOLUTION REACTION ON SURFACE OF MoB_2 DOPED BY TRANSITION METALS

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If hydrogen is ever to become the green fuel of the future, it is necessary to discover a cheap yet effective catalyst of Hydrogen Evolution Reaction (HER) which takes place in hydrogen fuel cells. We tried to find a suitable dopant for MoB_2 to increase its electrocatalytic activity for HER to approach this ideal catalyst. We used DFT calculations using VASP[1]–[3] to determine the adsorption energy of the hydrogen atom to the surface of MoB2 with or without dopant. We have studied the influence of ten different dopants from the Transition Metals - TM group (V, Cr, Mn, Fe, Co, Hf, Ta, W, Re, Os) on the Gibbs energy of adsorption of hydrogen (ΔG_{H^*}) as a marker of a effectivity of a catalyst[4], for four of them (Fe, W, Re, Os) we have also calculated the relationship between the energy of adsorption ΔG_{H^*} and surface coverage. Finally, we studied Tafel step of HER on pristine surface and surface doped by TM (Fe, W, Re) using *nudged elastic band*[5] and we have found out that ΔG_{H^*} alone may not be enough to evaluate the quality of a catalyst. To illustrate my work, I will present the results for pristine surface and surface doped with Fe and Os, which seem to be the best and the worst fit respectively.

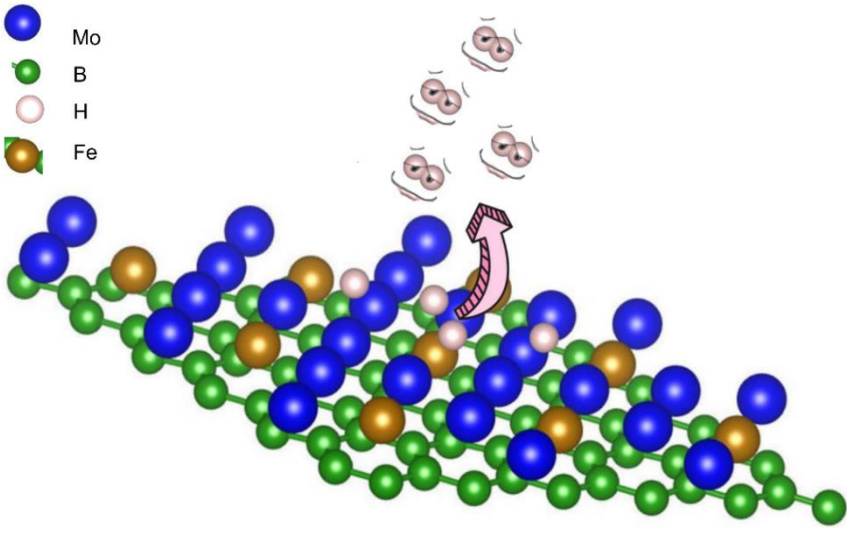

Figure: Hydrogen molecules desorbing after being relieved of their bond to the surface of MoB_2 doped with iron atoms.

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ELECTROCHEMICAL DETERMINATION OF INSULIN ON VARIOUS NICKEL MODIFIED ELECTRODES

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Since diabetes mellitus (DM) can be considered as one of the most crucial diseases worldwide there are more demands on the development of fast, effective, low cost and accurate sensors for early DM diagnosis due to the early treatment therapy [1]. Therefore, electroanalytical sensors for insulin determination can be represent the novel suitable tool for DM diagnosis. Electrochemical insulin detection takes only a few seconds and prepared electrochemical sensors are cheap and dispose of high sensitivity and selectivity [2]. Based on our previous research, nickel can be considered as the most suitable material for electrode modification due to its excellent catalytic activity towards insulin oxidation arising from the presence of NiOOH- in alkaline medium [3]. In our previous works we developed the electrode modified by Ni nanoparticles (NiNPs). The main disadvantage of mentioned modification was the worse reproducibility due to non-uniform nanoparticles distribution (Figure 1 A). Therefore we focused on the development of Ni modified electrode with Ni cavities prepared via colloidal lithography method, which provides much more uniform distribution (Figure 1 B).

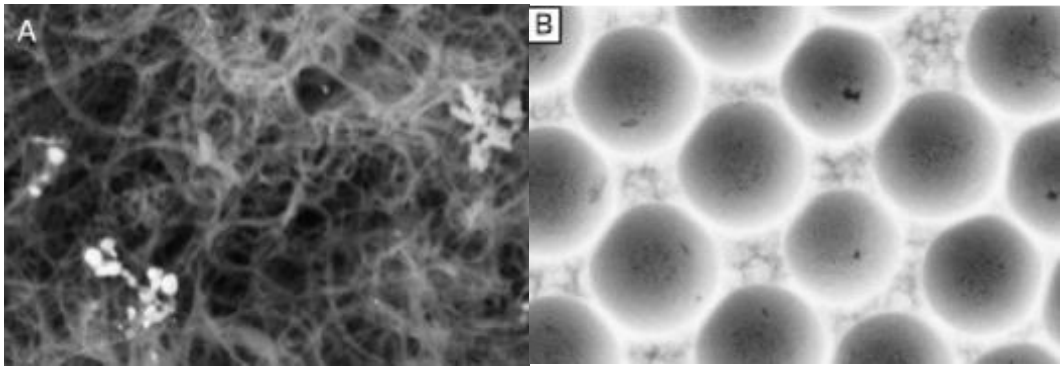

Figure 1: Non-uniform distribution of MWCNTs and NiNPs on the electrode surface (A) and uniform distribution of Ni-cavities on the electrode surface (B).

Both prepared electrodes Ni-cavities and NiNPs showed rapid enlargement of the surface area (2.89 cm^2-modified/1.87 cm^2-bare) and (0.77 cm^2-modified/0.3 cm^2-bare), respectively. Also the analytical characteristics of Ni-cavities modified electrode were determined usin cyclic voltammetr method. Prepared electrode was sensitive (1.032 μA μM-1) with low limit of detection (156 nM) and wide dynamic range (500 nM to 10 μM with R^2=0.99). Obtained characteristics were compared with carbon electrode modified by NiNPs. An electrode modified with nanoparticles displays a linear range of 250 nM to 5 μM (R^2=0.99), low limit of detection (94 nM) and a sensitivity of 0.021 μA/μM. Having in mind the possible interaction

between electrodes and interferences in human blood, the Ni-c-ITO was used for determination of insulin in real blood serum samples with any influence on insulin determination. Therefore, Ni-cavities modified electrode can be considered as the suitable candidate for further analysis as a platform for novel electrochemical sensors for diabetes diagnosis.

ACKNOWLEDGEMENT

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INTERACTION OF MERCURY IONS WITH HOMOTHYMINE STRETCHES IN DNA AT MERCURY ELECTRODE

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In addition to the natural way of base pairing in DNA using hydrogen bonds [1], the possibility of base pair formation via transition metal ions has been described. Thymine-thymine pairs can be formed, in which a mercury atom is bound between the nitrogen atoms in position 3 of the two thymine residues (Fig. 1). Similarly, cytosine pairs can be formed through silver ions [2]. The formation of T-Hg-T complexes has been used in the development of biosensors for detection of mercury based on monitoring the conformational changes in DNA after binding of the mercury atoms [3]. On the contrary, in this work, we used the possibility to monitor the representation of thymine residues in DNA adsorbed on the electrode surface by interaction with mercury ions generated directly by anodic oxidation of the material of the hanging mercury drop electrode (HMDE). Using cyclic voltammetry, peaks corresponding to reduction and oxidation of mercury ions were observed specifically for oligonucleotides containing homothymine stretches. In the case of oligonucleotides differing in the number of thymine residues (such as oligonucleotides derived from telomeric sequences of different organisms), mercury signals increased with increasing number of thymine residues, with already three thymine residues in the block sufficient to produce signals corresponding to mercury complex formation. Thus, this approach can be used both to detect the presence of homothymine stretches and potentially to estimate their length.

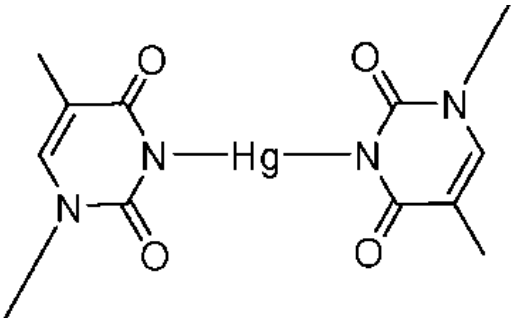

Figure: T-Hg-T complex

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ELECTROCHEMICAL PROPERTIES OF DRUG 3-FLUOROPHENMETRAZINE

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In recent years many new psychoactive substances have appeared in the drug market. These substances are usually not controlled by current legislation; therefore, they are used as a legal alternative to classical drugs. It is not easy to identify these compounds in organism, because there are lots of derivates.[1,2]

In the Czech drug market 3-fluorophenmetrazine (2-(3-fluorophenyl)-3-methylmorpholine, 3-FPM (Figure 1)) was first noticed in 2014. This fluorinated analogue of phenmetrazine can be bought over the Internet, it is sold in the form of powder, crystals, or pills. Phenmetrazine was well known under commercial name Preludin. In 1950s this substance was used for treatment of obesity. Adverse effects led to its withdrawal from the market. Preludin became popular in 1970s due to its association with The Beatles and replaced amphetamine at that time.[3,4,5]

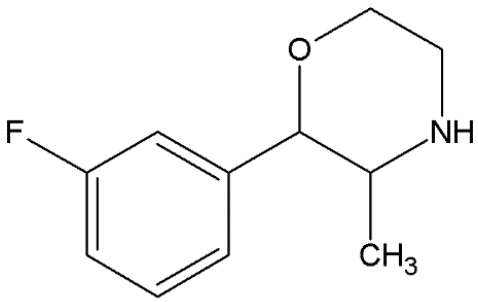

Figure 1: Chemical structure of 3-FPM

It is important to identify metabolic pathways of 3-FPM in human organism. Part of the reaction scheme is an electron transfer. According to a study by Mardal M. et al.[6] there N-oxide was found in human urine, other transformation pathways include aryl hydroxylation, O-methylation, alkyl hydroxylation, oxidation and degradation of the ethyl-bridge yielding the O/N-bis-dealkylated metabolite.

This study is focused on electrochemical and spectrophotometrical investigation of 3-fluorophenmetrazine. It is crucial to know reaction intermediates and products of oxidation or reduction for the final development of new efficient method for their detection. This report is based on cyclic voltammetry (CV), differential pulse voltammetry and UV-Vis spectrophotometry. The voltammograms were recorded at different pH values and different scan rate in aqueous medium. The glassy carbon electrode was used as a working electrode

for electrochemical measurements. The stability of 3-FPM was measured using UV-Vis spectrophotometry.

ACKNOWLEDGEMENTS

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BLOCKING THE NANOPORES - CONCEPTS AND MEDITATION ABOUT ELECTROCHEMICAL (LABEL-FREE) BIOSENSING

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We have previously proven that the output of the impedimetric immunosensor employing ferro-ferricyanide as redox probe is governed by the molecular charge of components involved [**Chyba! Nenalezen zdroj odkazů.**]. We dare to say that these principles and corresponding e ffects can be generalized for almost all comparable affinity-based electrochemical detection formats - the signal is governed mainly by the attractive and repulsive forces between interacting molecules at the electrode surface.

One of possible formats, where the mass transfer of a redox probe through the modification layers is governing the output signal (as it is regarded through the majority of the current scientific works in this field), is the blocking the nanopores on the electrode. Here, the effect of molecular charge is transferred from the electrode surface and only the physical blockage of the nanopore by the ligand (corresponding partner of immunoreaction) is observed [**Chyba! N enalezen zdroj odkazů.**]. Moreover, usage of nanopores represents a fundamental possibility of signal amplification in electrochemical immunoassays using only passive components (without any enzyme, quantum dot or another additional amplifying label).

Solid-state nanopores formed on top of the electrode surface can be realized through diverse ways. We prepared the nanoporous matrix using simple immobilization of spherical nonconductive (polystyrene) nanoparticles in dense monolayer – the open interstitial space among three neighbouring spherical NPs in the densest planar hexagonal arrangement forms a nearly triangular nanopore (Figure A, B). [2,3]

In our contribution, various shapes of nanopores and the causative effect on the generated signal will be discussed in general. We will summarize the effects mentioned above and resulting consequences including the presence of a mixed output signal (Figure C).

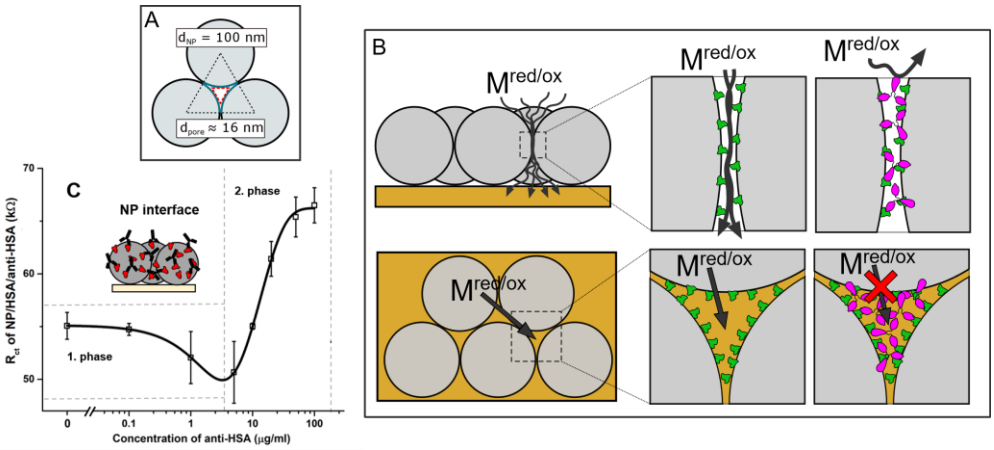

Figure A: approximate diameter of a nanopore formed by an alignment of spherical nonconductive nanoparticles, B: nanopore blockage with an antibody (the nanopore is modified with an antigen), C: mixed output of a nanoporous matrix modified with the antigen and the corresponding antibody, different influence of charge and mass-transfer effects at different distances from the electrode surface is apparent [2].

ACKNOWLEDGEMENT

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IS DIGITAL SCANNER SUITABLE AS CHEAPER ALTERNATIVE OF A SPECTROPHOTOMETER?

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Arrays and microarrays have become increasingly popular as tools for biological researches using different capture agents in arrays to detect DNA/RNA, proteins, carbohydrates, etc. [1]. This concept was also extended to identify and sense other chemical entities, e.g., ions, vapours, small organic molecules, etc. [1,2]. Technological advances such as device miniaturization combined with CCD technology (e.g., digital scanner/camera or cell phone) have enabled the measurement of experimental datasets effortlessly using readily available hardware and software tools other than a spectrophotometer in the lab [1].

This contribution demonstrates how the digital scanner could be employed for fast and routine analysis of some analytes in biological samples. The parameters for scanning utilizing a digital scanner have been optimized to treat the experimental data employing a newly developed software ScanQuant2. The determination of Cu (II) and ammonium ions with commercial analytical strip papers and 96-well plates using a digital scanner shows that it can be employed as a suitable alternative to the classical spectrophotometric approach. In addition, a methodology developed for the analysis of ammonium ions was also utilized for the enzymatic determination of urea.

ACKNOWLEDGEMENT

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KINETIC STUDY OF CU(II) COMPLEXES OF MONO- AND BIS-TETRAAZAMACROCYCLIC LIGANDS

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Polyaza-macrocyclic ligands have been recently used to bind some radioisotopes for application in medicinal chemistry ($^{60–64,67}$Cu, $^{66–68}$Ga, ^{86,90}Y, ^{111}In) [1,2]. These metal complexes have to exhibit high thermodynamic stability and kinetic inertness for possible *in vivo* use for medical purposes [1, 2]. Several copper(II) and zinc(II) complexes of bis-polyaza-macrocyclic ligands catalyse some hydrolysis-like chemical reactions and therefore they can be used as model systems mimicking enzymes activity [3].

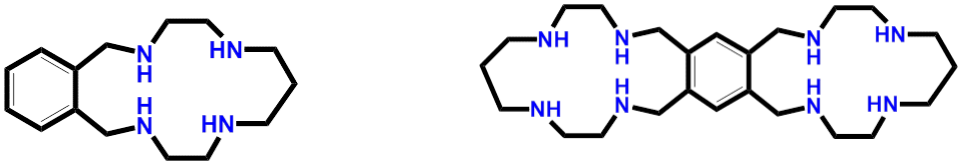

Figure: The structural formulas of studied macrocyclic ligands

The thermodynamic studies of Cu(II) complexation by both ligands (see Figure) show that these complexes are stable [4-6] due to fact that copper(II) ion is fully coordinated by all four nitrogen donor atoms [4-6]. In this work, the formation of the copper(II) complexes of both ligands was studied as function of pH and possible reaction mechanism is proposed. The study of acid-assisted dissociation of both copper(II) complexes does not differ within experimental error. The results for both formation and dissociation of Cu(II) complexes indicate that there is no cooperative effect for binucleating ligand.

ACKNOWLEDGEMENT

The work has been supported by Masaryk University (MUNI/A/1192/2020), Ministry of Education of the Czech Republic (LTC20044) and EU (COST CA18202 NECTAR Action).

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PLANT PHENOTYPING: IN-HOUSE BUILT CAPILLARY ELECTROPHORESIS DEVICE FOR "IN-VIVO" ANALYSIS OF PLANT SAPS

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Developing plant phenotyping techniques includes analyses of plant intercellular matrix (apoplast) composition. Our approach consists of a semi-invasive sampling method combined with Capillary Electrophoresis (CE) based methods using different types of detection.

The CE analytical method provides many advantages emphasizing short analysis time, high separation efficiency, sensitivity, and very low volumes [1]. All these features enable us to apply CE methods, also in the case of living plant analyses, where the sample is limited to sub microliter volume [2]. Additionally, the CE-based methods can be coupled with the MS detection, which makes them complementary to LC-MS, esp. regarding plants' analytes with low endogenous concentration [3].

In this work, regarding plant phenotyping, the focus is on developing novel instrumentation for the analysis of various ionogenic metabolites and plant hormones within the *Brassicaceae* family.

The device under development in our laboratory aims at analyzing chosen analytes in plant saps during their development or stress response.

The main part of this work is a made-to-measure fabricated device for performing capillary electrophoresis (CE) techniques. The device utilizes two types of detection, i.e., UV detector and Capacitively-Coupled Contactless Conductivity Detector (C4D). The base plate of the device is attached to the top of the UV detector while surrounding the UV detection cell.

On the base plate, two vessels for the background electrolyte are mounted, and on the inlet side a swinging arm. The arm serves for fixing and precise positioning of the sampling end of the capillary. The capillary is further led through the C4D cell, followed by the UV detection cell.

A method for the separation of a group of plant hormones – auxins – was successfully applied and optimized with the use of this device. Only naturally occurring auxins were chosen, i.e. indole-3-acetic acid (IAA), 2-phenylacetic acid (PAA), indole-3-butyric acid (IBA), and indole-3-propionic acid (IPA).

The analytes are chemically organic acids; therefore, they are separated as anions. For this purpose, a separation method of Capillary Zone Electrophoresis (CZE) based on [4], as well as a method using polyacrylamide-coated capillary as in [5], were examined.

The composition of a background electrolyte (BGE) was chosen regarding possible MS detection to avoid the addition of surfactants for altering the electroosmotic flow (EOF). Various co-ions and pH values were tested for determining the best condition for separation.

As a result of optimization, a separation condition consisting of 20 mM ammonium acetate pH 6.9 was chosen. The condition was performed in a polyacrylamide-coated capillary with an inner diameter of 75 μm, using separation voltage of 15kV (300 V/cm) - negative polarity. Concerning the detection, both detectors were properly adjusted, C4D for the composition of the chosen BGE, and also UV detection wavelength was determined from measured spectra of

analytes. Samples were injected hydrodynamically, the cathodic end was elevated to a height of 2.5 cm for 50s.

The next step will be the application of this method for analyzing auxins within plant sap. Plants of our interest within the *Brassicaceae* family are mainly *B. napus* and *B. rapa* (oilseed rape), but also other crops from this family. According to the values of auxins concentration in the crops within *B.* family species, ca. µmol – pmol/g Dry Weight (DW) [6], MS detection will be most likely engaged for enhancing the sensitivity.

The group of four naturally occurring auxins in the plant sap were successfully separated using an in-house built CE instrument.

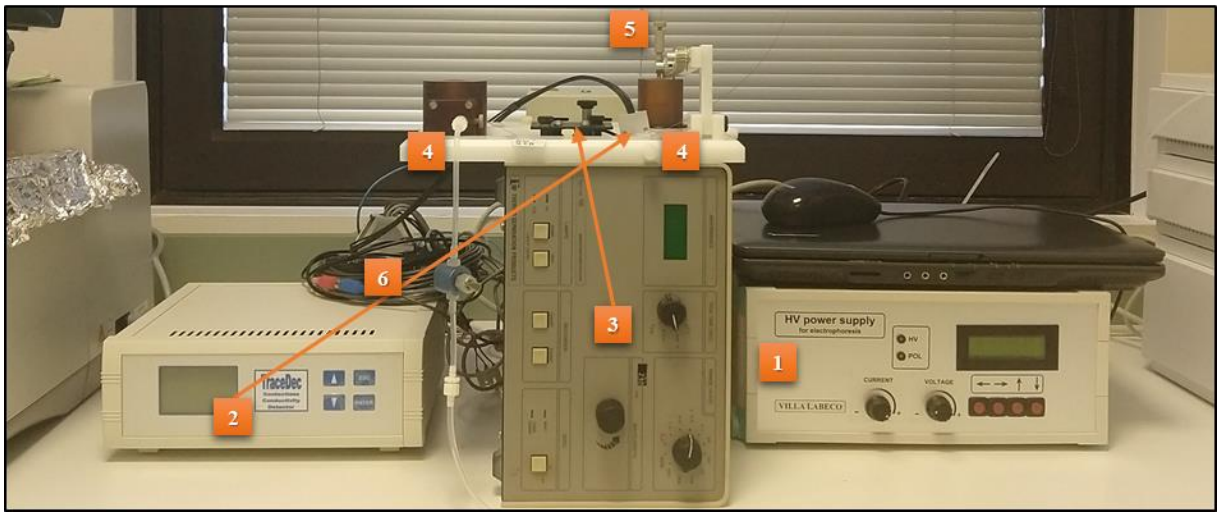

Figure 1: In-house built CE device 1 – HV power supply; 2 - C4D; 3 – UV detector; 4 – vials + electrodes; 5 – fused silica capillary; 6 – DAQ.

ACKNOWLEDGEMENT

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VOLTAMMETRIC DETECTION OF 8-HYDROXYGUANINE AT THE SPCE MODIFIED WITH MWCNTS-COOH

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In this contribution, the use of screen-printed carbon sensors modified with multi-walled carbon nanotubes functionalized with carboxy groups (MWCNTs-COOH) for the electrochemical detection of 8-hydroxyguanine (8-OH-Gua) is presented. First, three-electrode screen-printed sensors DRP-150 and DRP-C110 with unmodified carbon working electrodes and DRP-110CNT, modified with MWCNTs-COOH (all Metrohm Czech Republic), were tested using a solution of 200 μmol dm^{-3} 8-OH-Gua. The highest current response was achieved on the DRP-110CNT sensor, which was then used for further experiments. The position and intensity of the oxidation peak were significantly influenced by the pH of the supporting electrolyte. The highest current signal with peak potential at +0.26 V was recorded in solution with pH 7. The dependence of the peak potential vs. pH was linear with a slope close to theoretical value of 0.0592 V pH^{-1}, indicating an electrochemical process involving the same number of protons and electrons.

The determination of 8-OH-Gua using a screen-printed carbon sensor modified with MWCNTs-COOH was then validated. A linear calibration plot was constructed within 0.3–12.0 μmol dm^{-3} 8-OH-Gua (Figure 1) and LOD of 0.57 μmol dm^{-3} and LOQ of 1.89 μmol dm^{-3} were calculated. Also, it is possible to assay 8-OH-Gua in an extended linear range from 0.3 to 240 μmol dm^{-3} with a bit lower sensitivity. Further improvement of analytical performance is possible by using square-wave voltammetry, which is currently under investigation. The accuracy and precision of the intraday and interday assays of 8-OH-Gua samples were evaluated at three concentration levels: low concentration of 2 μmol dm^{-3}, medium concentration of 20 μmol dm^{-3}, and high concentration of 200 μmol dm^{-3}. Measurements were made under the same conditions at the same concentrations three times (n = 6) in 1 day for the intraday assay and once a day (n = 6) during three consecutive days for the interday assay. The intraday assay accuracy values were 101.47%, 99.87% and 100.02% at low, medium and high concentrations of 8-OH-Gua, respectively. The average intraday precision values ranged from 5 to 12%. The accuracy values for the interday assay were 83.60%, 101.89% and 100.07% for low, medium and high concentrations, respectively. The average precision between days was within 5 to 14%. The stability of the 8-OH-Gua solution was also monitored when stored in the refrigerator at 4 °C, in freezer at −20 °C, and at laboratory temperature of 21 ° C [1].

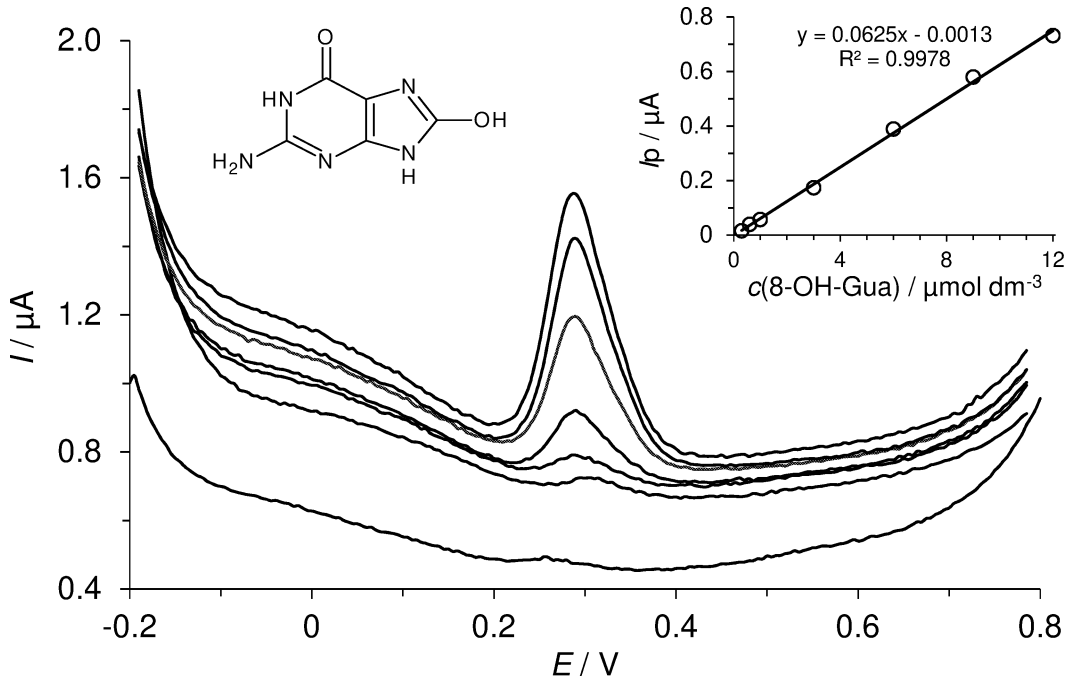

Figure 1: DPV voltammograms of 0.3–12.0 µmol dm^{-3} of 8-OHGua in 0.01 mol dm^{-3} PBS (pH 7) using the DRP-110CNT sensor together with the corresponding calibration line (inset).

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ELECTROCHEMICAL ASSAY FOR DETECTION OF PROSTATE CANCER BIOMARKERS IN URINE

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Current molecular diagnostics of prostate cancer is based on monitoring elevated levels of prostate-specific antigen (PSA) protein in serum. This single biomarker, however, can lead to unnecessary biopsies due to its low specificity for prostate cancer. Recently approved long non-coding RNA (lncRNA) biomarker *prostate cancer antigen 3* (PCA3), combined with PSA determination, offers a better distinction between prostate cancer and non-malignant prostate diseases [1]. PCA3 levels are determined from prostate cells in urine after digital rectal examination and compared to PSA mRNA levels.

We applied this clinically used principle to develop an electrochemical assay for determination of PSA and PCA3 on RNA level from urine. Based on our recent works that targeted HPV DNA in cervical cancer [2, 3], here we combined LAMP reaction, which is an isothermal amplification technique showing high sensitivities at constant temperatures and shorter reaction times, with hybridization at magnetic beads and chronoamperometric detection at carbon electrode chips. We reached good sensitivity and specificity for both biomarkers in prostate cancer cell lines. Ultimately, we demonstrated our approach in clinical samples, i.e., urine samples from 11 prostate cancer patients and 7 healthy controls, where we measured both biomarker levels. Their ratios (PCA3/PSA) showed up excellent correlation with clinical data.

ACKNOWLEDGEMENT

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VOLTAMMETRY OF SUDAN I AT PYROLYTIC GRAPHITE AND BORON DOPED DIAMOND ELECTRODES IN AQUEOUS MEDIA

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Sudan dyes are synthetic azo-based aromatic compounds, traditionally used in waxes, plastics, oils and polishes. They have been categorized as class 3 carcinogens by the IARC [1]. Sudan I is a dye used as an orange colouring agent. In mammalian organisms, Sudan I is metabolized by the microsomal detoxifying system with a central role of cytochrome P450 (CYP) hydroxylation activity [2]. Electrochemical methods have been applied to determine Sudan I in relevant matrices, with particular attention paid to analysis of food samples.[1,3] The dye can be detected either via oxidation of a phenolic group, or via reduction of an azo group present in its molecule. These primary conversions result in formation of other electrochemically active moieties.

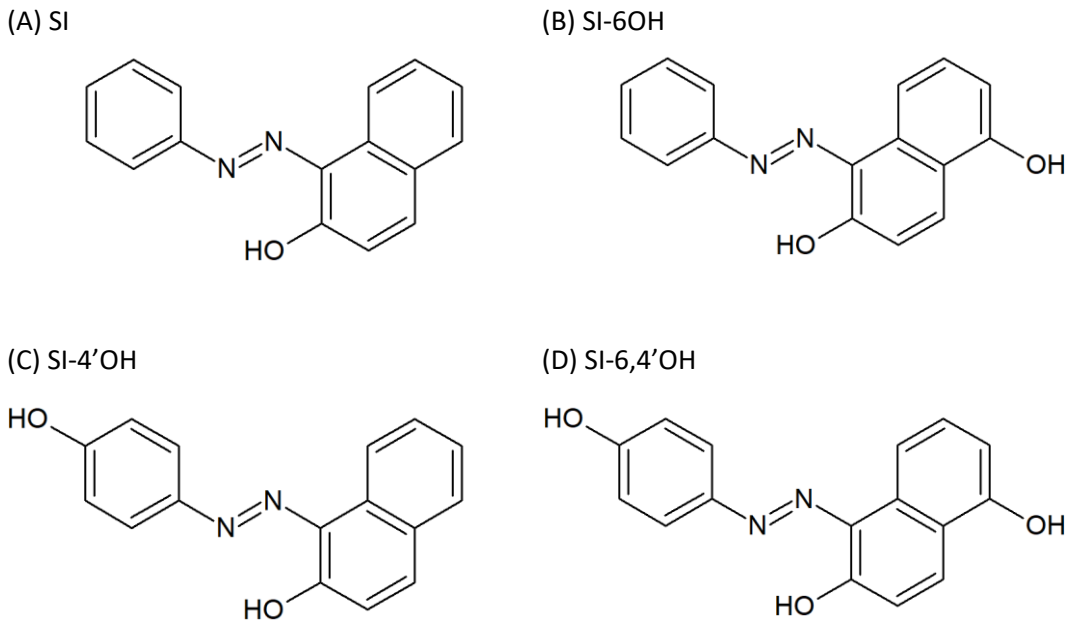

Figure: Chemical structures of Sudan I and its hydroxylated metabolites: (A) Sudan I, (B) 6-OH Sudan I, (C) 4'-OH Sudan I, (D) 4',6-diOH Sudan I.

In this work we present a comparative study of Sudan I redox processes at pyrolytic graphite and boron doped diamond electrodes in aqueous buffer solutions as well as its three hydroxylation derivates (Figure 1). We focus on adsorption of the analyte and products of its electrochemical conversion at the two electrodes surfaces to find optimum conditions for

efficient voltametric detection of the dye and the possibility to differentiate SI from its derivates in solution.

ACKNOWLEDGEMENT

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EFFECT OF SILK FIBROIN ON DIFFUSION PROPERTIES OF AGAROSE HYDROGELS

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Silk fibroin is a protein derived from silkworm (B. Mori) cocoons and it is a unique material widely used for its properties (toughness, mechanical strength, biocompatibility, and low immune response) in medical and tissue engineering applications. Also, agarose, a linear polysaccharide derived from seaweed, is a biocompatible material that forms a hydrogel, widely used in the same applications. Extracellular matrix, the basis for living cells, consists of two parts – fibrous (elastin, collagen) and amorphous (PG's, GAG's), so it has the character of hydrocolloid. However, it is a structurally very complex material, so for the experimental part, simple hydrogels were formed, which also consist of a fibrous component, silk fibroin, in an amorphous component, an agarose hydrogel.

Silk fibroin was extracted from raw silk fibres using Ajisawa's reagent [1]. Samples contained agarose and silk fibroin, both in two different concentrations (0,5 and 1,5 wt. % for agarose and 2,0 and 5,0 wt. % for silk fibroin), also samples without fibroin were prepared. The diffusion model of the constant source is a simple macroscopic observation of diffusion of methylene blue (MB) from solution (0,01 g/l MB) into cuvettes containing agarose and fibroin samples. At defined time (24, 48 and 72 hours) the concentration of MB in dependence on the distance from the hydrogel/solution interface was determined using UV-VIS spectrometry (Cary50, Varian). Then theoretical models and MB concentration profiles were obtained, and the values of the effective diffusion coefficient were determined.

In all samples, the diffusion of MB was slower than in water. For samples consisting only of agarose, the effective diffusion coefficient of MB decreases with increasing agarose concentration. In samples with fibroin, the diffusion rate of MB decreases with increasing fibroin concentration. The fastest diffusion was observed in 0,5 wt. % agarose, then the same concentration of agarose with 2,0 wt. % fibroin, followed by 1,5 wt. % agarose and 1,5 wt. % agarose with 2,0 wt. % fibroin, and the slowest diffusion was observed in samples that contained 5,0 wt. % fibroin (for both 0,5 and 1,5 wt. % agarose).

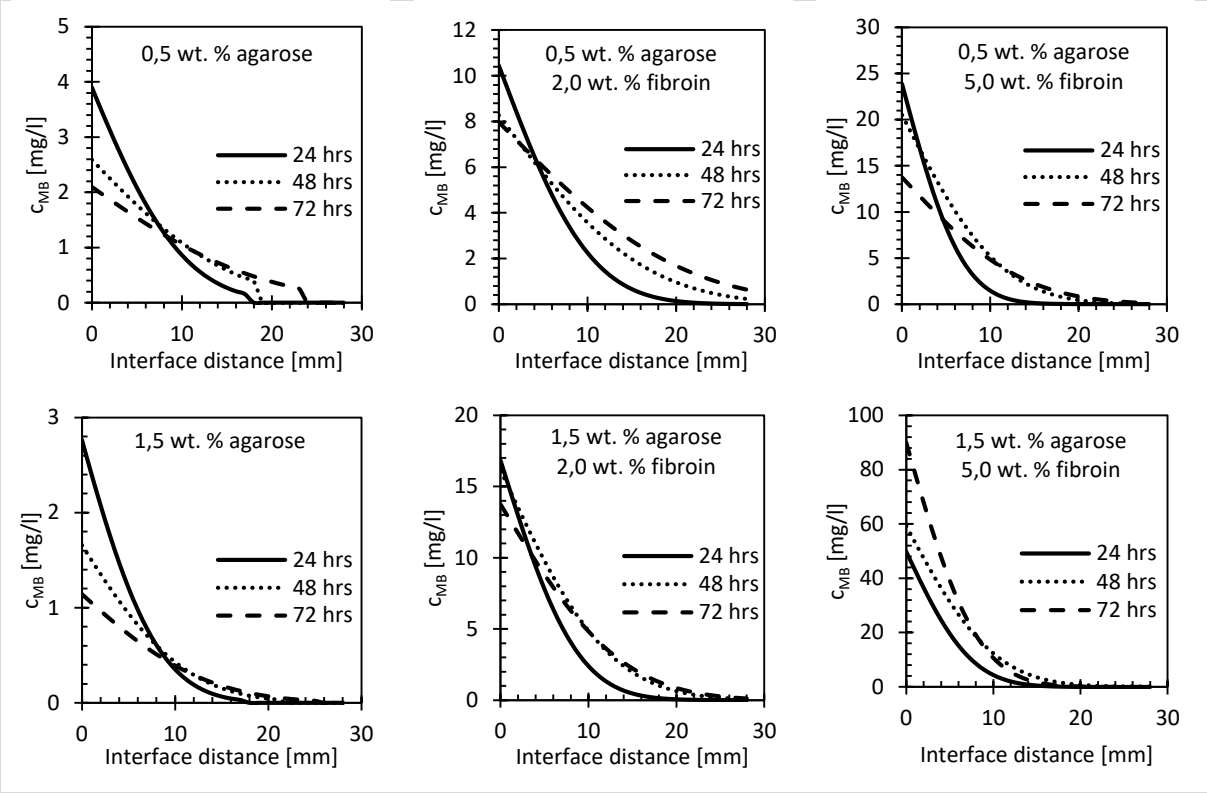

Figure: Concentration profiles of methylene blue (concentration of methylene blue in dependence on the distance from the hydrogel/solution interface) in agarose hydrogels (0,5 and 1,5 wt. %) pure and with silk fibroin (2,0 and 5,0 wt. %).

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AFRICAN SWINE FEVER VIRUS DETECTION USING SUPERMAGNETIC NANOPARTICLES

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INTRODUCTION

African swine fever (ASF) is a very serious disease that affects members of the Suidae family. ASF first appeared in the sub-Saharan region of Africa [1], and from there the disease spread across the Transcaucasian region to Europe and Asia. There is a growing concern that the disease will spread to other areas because of travel and imports of pork products from countries where ASF is present. The causative agent of ASF is an enveloped cytoplasmic double-stranded DNA arbovirus (*Asfavirus*) (genome size 170–193 kbp), which is the only member of the Asfarviridae family. The natural cycle of ASF virus (ASFV) spread occurs only in some parts of Africa and in the Iberian Peninsula. The first case of an outbreak of ASF in China was reported at a suburban pig farm in Shenyang in 2018. The low concentration of viruses in environmental samples makes detection extremely difficult. Therefore, simple, accurate and fast detection methods are urgently needed. Portable detectors based on biological molecules could be very helpful for rapid diagnosis in an outbreak. The aim of this work is to verify the possibilities of direct capture of DNA on magnetic nanoparticles and subsequent detection of DNA using electrochemical methods.

MATHERIALS AND METHODS

All chemicals used in this study were purchased from Sigma-Aldrich (St. Louis, MO, USA), in ACS purity. The resistance of deionised water used for this research was 18 MΩ. Electrochemical measurements were performed with AUTOLAB Analyser connected to VA-Stand 663, using a standard cell with three electrodes. The working electrode was a hanging mercury drop electrode (HMDE) with a drop area of 0.4 mm^2. The reference electrode was an Ag/AgCl/3M KCl electrode, and the auxiliary electrode was a graphite electrode. Gold-coated superparamagnetic iron oxide nanoparticles (Au-SPIONs) were prepared [2]. DNA of ASFV

was selectively captured on Au-SPION, therefore in a sample only DNA of ASFV was present. DNA was detected using electrochemical methods in combination with adsorptive transfer technique (AdT). Two methods were used: differential pulse voltammetry (DPV) – the supporting electrolyte was containing 0.2 M acetate buffer (pH 7.0) – and chronopotentiometric stripping analysis (CPSA) – the supporting electrolyte containing 0.2 M McIlvaine's buffer (pH 5.9) was used. Parameters of CPSA were the following: time of accumulation 120 s, stripping current 20, 10, 5, 1 µA, measurement time 240 s.

RESULTS AND DISCUSSION

Electrochemical methods have been used for nucleic acid (NA) analysis for over 50 years. In our work, a strategy for detecting the presence of ASFV using DPV (DNA signal and quantum dots) was proposed [3]. In addition to redox NA signals, NAs also provide catalytic signals. A suitable analytical method for the catalytic signal (peak H) is CPSA. We focused on the description of the basic behavior of the H peak induced by the presence of dsDNA (in McIlvaine's buffer , pH 7.0). Peak H signal appeared at potential -1.75 V. We verified that the presence of DNA in the sample leads to the catalytic signal of the H peak (n = 10). We further studied under which conditions (concentration of DNA in the sample, applied current used for analysis and maximum duration of analysis) different catalytic signals are generated and how the H catalytic peak of DNA changes. The applied current is essential for the subsequently detected dt/dE signal. The applied current suitable for analysis varies according to the amount of dsDNA present in the sample. We tested different applied currents (20, 10, 5 and 1 µA). At a DNA concentration of less than 1 µg/mL, we achieved the best-plotted H peaks (dt/dE) at a current of 5 µA. For DNA concentrations of 1, 5 and 10 µg/mL, a current of 10 µA was applied and for concentrations of 10, 15 and 20 µg/mL, a current of 15 µA was applied. For DNA concentrations above 20 µg/mL, the best responses were obtained when applying a current of 20 µA. Sufficient analysis duration (dependence of a potential E on a time t) is required to fully record the signal of the H peak of dsDNA at a given applied current. Extending the DNA analysis time at a given current significantly improved the dt/dE records of the H peak and the reproducibility of the analysis (RSD up to 10%). It was found that the suitable time for which the H peak is reliably plotted is around 240 s.

CONCLUSION

The combination of Au-SPIONs and electrochemical detection provides a very useful tool for detecting the presence of DNA in very low concentrations. The procedure was validated to detect the presence of ASFV DNA with 100% sensitivity and selectivity.

ACKNOWLEDGEMENT

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SCREEN-PRINTED ELECTRODE BIOSENSORS FOR DETECTION OF CANCER BIOMARKERS

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DNA or RNA biosensors based on screen-printed electrodes (SPE) have been a promising strategy for detection of cancer biomarkers such as DNA mutations, microRNAs, oncoviruses, and epigenetic modifications [1] Herein, we present applications of SPEs for detection of human papillomavirus (HPV) that cause several anogenital malignancies, especially cervical cancer in women, as well as for detection of *KRAS* and *BRAF* gene mutations important in various cancers, e.g. colorectal or pancreatic cancer.

For the HPV detection, we have designed electrochemical biosensor that aims to capture two most frequent high-risk HPV types, HPV16 and HPV18. Instead of using magnetic beads as in our previously published works [2-4], we directly modified the screen-printed gold electrode (SPAuE) with 6-mercapto-1-hexanol as backfiller along with thiolated capture probe specific for either HPV16 or HPV18 DNA. To amplify viral DNA, we used isothermal amplification technique called LAMP (loop-mediated amplification) which is faster than PCR and does not require thermal cycling. LAMP products contained biotinylated dUTP nucleotides that then bound to streptavidin-horseradish peroxidase conjugates for amperometric readout of the enzymatic reaction. We addressed the issue of sensitivity, selectivity, stability as well as cross-reactivity, and we obtained satisfactory results on cervical cancer cell lines CaSki and SiHa (HPV16-positive), HeLa and C4-I (HPV18-positive). Further experiments will focus on application of the biosensor into clinical samples.

We also focused on utilizing either SPAuEs or screen-printed carbon electrodes (SPCEs) for analysis of DNA mutations in *KRAS* and *BRAF* genes, using various colorectal cancer cell lines and also various amplification techniques, including PCR, LAMP etc. For instance, the PCR-based approach on SPCEs distinguished between wild type and mutation-containing cell lines for both *KRAS* and *BRAF* gene, however, more work is still necessary before its application to the clinical samples.

ACKNOWLEDGEMENT

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SILICA NANOFLUID PHYSICAL CHARACTERIZATION AND ITS INTERACTION WITH PROTEINS

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These days, nanoparticles are intensively studied by many researchers mainly due to their attractive properties and broad range of application. Not only high surface-to-volume ratio but also size, great biocompatibility, and surface charge make them interesting for application in nanomedicine, diagnostics, cosmetics, printable electronics etc. Also, the intracellular uptake is higher in comparison with microparticles [1].

It is important to study physical properties of nanoparticles because they get into the biological environment either intentionally, as drugs in pharmaceutical industry, or accidentally in textile industry or as metabolic products. When the nanoparticles come into contact with body fluid, they are immediately covered by proteins – protein corona is formed and consists of two parts, hard and soft. This is very important process to be studied because it affects cellular uptake and, therefore, subsequent fate of nanoparticles *in vivo*.

In this work, LUDOX® colloidal silica nanofluid of three different size, 7 nm (SM30), 12 nm (HS40) and 22 nm (TM40), was characterized by a bit unusual methods – densitometry and high-resolution ultrasonic spectroscopy (HRUS). Colloidal silica is fine amorphous, nonporous spherical nanoparticles suspended in water, so-called nanofluid. Also, the effect of temperature (25 and 37°C) and environment (water and PBS) was investigated to see if there is any change in density or compressibility for potential application of the nanoparticles in pharmaceutical industry.

Nanofluid density in concentration range from 0 to 30, resp. 40 wt%, was studied by DSA 5000 M Density and Sound Velocity Meter. Ultrasound velocity and attenuation of the nanofluid was measured by HRUS. It is a non-destructive real-time method to study intrinsic properties of liquid using ultrasound wave. Combining these two methods allow us to calculate nanofluid compressibility, an important property for further application. Bovine serum albumin (negatively charged at neutral pH and) and lysozyme (positively charged at neutral pH) were used as model protein to mimic biological environment to study the interactions between nanoparticles and proteins. These interactions were investigated by various methods – isothermal titration calorimetry (ITC), dynamic light scattering (DLS) titration and HRUS titrations.

The results of density measurements confirmed general knowledge of the effect of particles addition – density increases linearly with nanofluid concentration. There was a negligible effect of temperature and environment on nanofluid density, regardless LUDOX® type. There was no sedimentation of the sample because ultrasound attenuation remained constant during measurement (20 min). *Fig. 1* shows ultrasound attenuation (dU) of LUDOX® TM40 as a function of nanofluid concentration. The minimum indicates some kind of interaction between nanoparticles or with water in terms of change in hydration layer. Also, nanofluid apparent characteristics were calculated, such as apparent specific volume V_{app}, partial specific volume

V_{sp} and apparent compressibility $\square_{app}$. Furthermore, calculation of nanofluid compressibility shows that nanofluid compressibility decreases with increasing nanofluid concentration.
DLS titration of proteins into nanofluid are in a good agreement with results from ITC titrations – is shows no interaction of BSA with nanoparticles and classical sigmoidal curve for interaction between lysozyme and nanoparticles.

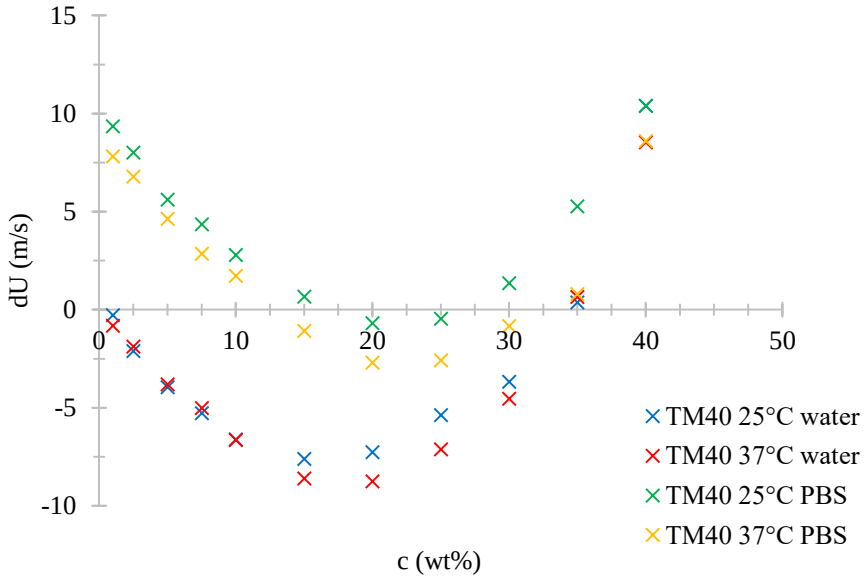

Figure: Representative graph of ultrasound attenuation (dU) of LUDOX® TM40 nanofluid in PBS and water at 25 and 37°C as a function of concentration.

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A COMPUTATIONAL STUDY OF Pb(II) COMPLEXES WITH TETRAAZAMACROCYCLIC LIGANDS

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$^{203/212}$Pb(II) macrocyclic complexes have potential in cancer theranostics as cytotoxic α emitters [1,2]. In order to utilize these complexes *in vivo* and for any other applications, their behavior in various conditions must be understood. This work first experimentally examines the thermodynamic and kinetic properties of a series of Pb(II) complexes with tetraazamacrocyclic-based ligands. The rates of formation and dissociation of these complexes in varying conditions were monitored using UV-Vis spectroscopy. The most thermodynamically stable complexes with Pb(II) are formed with a ligand with four acetate-based pendant arms, while the most thermodynamically labile complexes have a larger or smaller cavity or phospinic-acid based pendant arms. The complexes with the fastest rates of formation are those with phosphinic-acid based pendant arms or a smaller cavity. The mechanisms of formation and dissociation of these complexes are also proposed.

This work further examines the investigated complexes using computational chemistry. DFT calculations using the PBE0 functional with the Def2TZVP basis set were used to optimize the geometries of the complexes and calculate parameters such as bond length. The AIMAll program was also used to calculate the delocalization indexes of the optimized complexes. These values were then correlated with experimental results, such as stability constants and rates of formation/dissociation, and certain trends were observed. Complexes with longer Pb-N bond lengths and lower complex energies have faster rates of formation, while complexes with longer Pb-O bond lengths and a higher delocalization index have enhanced kinetic inertness, a larger stability constant, and a longer complex half-life. This information can be used for the rational design of ligands having desired properties for the specific application of interest.

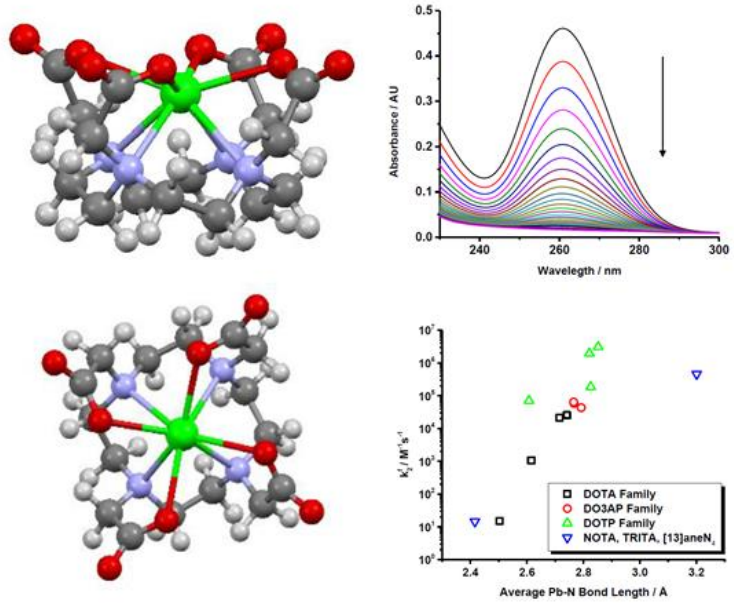

Figure*:* a) calculated structure of the Pb(II)-DOTA complex, b) dissociation of the Pb(II)-DOTA complex in 1M $HClO_4$, and c) graph showing the correlation between the formation rate constant k^2_f and the average Pb-N bond length for all investigated complexes.

ACKNOWLEDGMENTS:

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COBALT NANOPARTICLES MODIFIED SCREEN PRINTED ELECTRODES AS A NOVEL PLATFORM FOR INSULIN DETERMINATION

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Various transition metal nanoparticles represent promising low-cost materials for catalysis of insulin oxidation. Combination of transition metal nanoparticles with multi walled carbon nanotubes (MWCNTs), ensure the enlargement of the active surface area of the electrode, can improve the analytical characteristics of electrodes in many ways [1]. Usage of polymer membrane on the electrode surface prevents the occupation of active sites with Cl^- ions present in body fluids and improves the stability of the electrode by fixing the nanoparticles on the electrodes surface during the electrochemical measurements [2]. In this work screen-printed carbon electrodes (SPCEs) modified by the combination of copper (CuNPs) polymer membrane (chitosan) and MWCNTs was prepared. The surface of prepared electrode was studied via atomic force microscopy (AFM) and scanning electron microscopy (SEM) (Figure 1). In the effort to find the most suitable modification for electrochemical insulin determination, stability, analytical characteristics, and selectivity of the electrode was determined. The results proved low limit of detection (25 nM), high sensitivity (0.031 mA μM^{-1}) and wide linear range (0.05 μM to 5 μM) of CoNPs/chitosan-MWCNTs/SPCE. The stability of the CoNPs/chitosan -MWCNTs/SPCE was very high, with only 1.7% decrease of maximal current value after 50 measurements. Therefore, CoNPs/chitosan-MWCNTs/SPCE can be considered as the suitable modification for new electrochemical sensor for insulin determination, with suitable analytical characteristics.

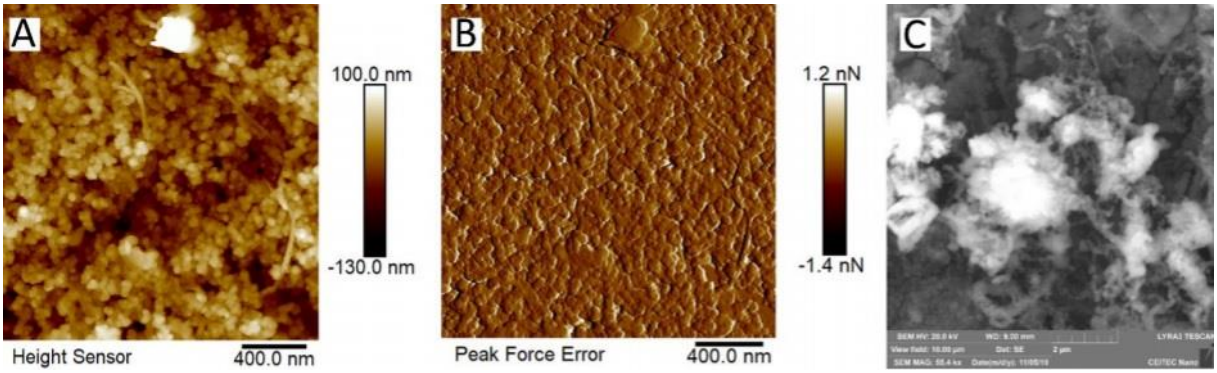

Figure 1 AFM image s of CoNPs/chitosan -MWCNTs/SPCE (Figure 1 A, B), detailed SEM image s of CoNPs/chitosan -MWCNTs/SPCE (Figure 1 C)

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UNIQUE ELECTROCHEMICAL PROPERTIES OF POLYMER PENCIL GRAPHITE LEADS

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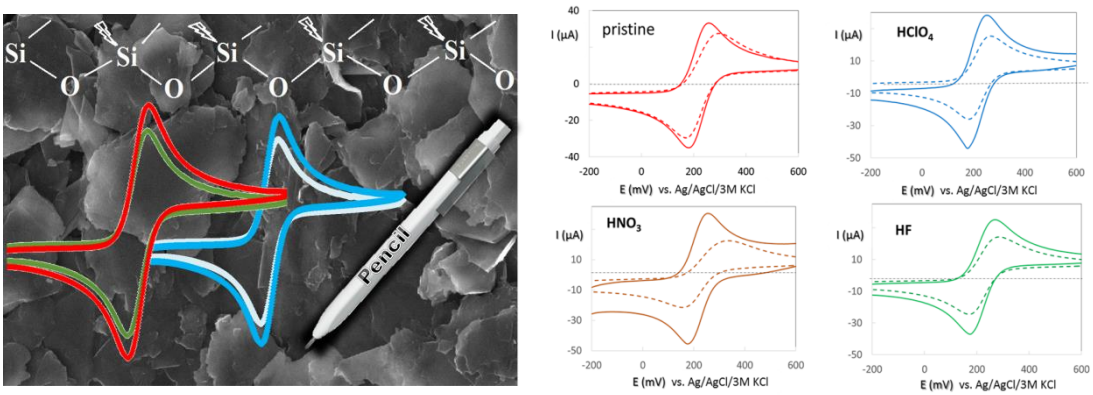
When, in 1795, Jaques Conte made the first mechanical pencil leads from graphite powder and clay, he had no idea that graphite pencil would become not only a writing tool but also a successful electrode material with unique electrochemical properties. Our research showed that pencil leads marked as "polymer" exhibit excellent electrochemical properties, such as a large potential window in both, the cathodic and anodic direction, a high signal-to-noise ratio and low background currents, and high electron transfer rates. We have found that the polymer pencil graphite electrode (pPeGE) is the best graphite sensor for the oxidation processes of numerous analytes (e.g., purine derivatives, amino acids, biopolymers) compared to other unmodified graphite electrodes [1-6].

The aim of this work was to answer the questions: (a) what is the surface morphology of our pencils and what polymer forms their nanocomposite surfaces, (b) why the electron transfer kinetics is fast and background currents are low, and (c) what the resistance of pPeGE surface to inorganic acids and organic solvents is. The chemical and structural behavior of pencil leads after exposure to acids (HF, HNO_3, $HClO_4$) or organic solvents (CH_3CN, CH_3Cl) was monitored *via* X-ray photoelectron spectroscopy (XPS) and scanning electron microscopy (SEM). The electrochemical activity of pristine and chemically and thermally treated pPeGEs was studied by the cyclic voltammetry (CV) of reversible redox probes $[Fe(CN)_6]^{3-/4-}$ and $[Ru(NH3)_6]^{3+/2+}$ [7].

The XPS experiments provided two essential results: the majority of sp^2 hybridized carbon atoms and the presence of polysiloxanes in the pPeGE surface layer. The presence of siloxanes was also demonstrated by exposure of the electrodes to chloroform, where the Si peaks in XPS decreased significantly. The SEM experiment revealed carbon scales and wrinkled surface morphology. Voltammetric experiments showed relative stability of pPeGE surfaces documented by differences in the potentials (ΔE_p) of the anodic and cathodic peaks of redox probes. However, significant differences in ΔE_p values were observed for the dried electrodes (200 ^oC, 24 hours). The comparison of pristine and heat-treated pPeGEs indicated that surface water improves the electrode performance by (i) increasing the conductivity, (ii) reducing the value of the charging current, and (iii) accelerating electron transfer [7]. Generally, siloxanes, due to their hydrophobicity and chemical composition, may aid the penetration of water into the pores of wrinkled and scaly pPeGE surfaces. It was found that surface water facilitates electron transfer, producing higher electrical conductivity and lower background currents.



Figures: The graphical abstract demonstrating the scaly pencil surface and draws attention to surface polysiloxanes. The impact of surface water on the ΔE_p ($[Fe(CN)_6]^{3-/4-}$ in 0.1 M KCl): the cyclic voltammograms of the non-dried (full lines) and dried (dashed lines) pPeGEs, pristine and chemically treated electrodes. The scan rate was 200 mV/s.

The presented characterization of pPeGEs will lead not only towards a deeper understanding of the electrochemical processes but also the development of advanced electrochemical applications, including sensors and batteries.

ACKNOWLEDGMENT

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ELECTROCHEMICAL AND SPECTROSCOPIC ANALYSIS OF INSULIN

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Insulin has been at the forefront of scientific interest for many years. It often serves as a model polypeptide in biochemistry, pharmacology, cell signaling, structural biology, and medicine [1]. Recently, this hormone that regulates blood glucose levels is often mentioned in connection with Covid-19 disease, as it has been found that patients with diabetes mellitus (DM) have a significantly higher risk of the disease. In the context of these findings, researchers are trying not only to develop a low-cost sensor with a rapid response to insulin [2], but also to know how insulin behaves in solution and at the charged phase interface, how its structural properties change depending on the external environment, and which amino acids are responsible for its redox processes.

Here, we present the latest results of electrochemical and spectroscopic studies of insulin supplemented by theoretical calculations of structures (molecular dynamics) [3,4]. Oxidation processes on screen-printed graphite electrodes (SPGE), nanostructured electrodes with bismuth (BiNP), or carbon nanofibers (CNF) reflect the conformational dynamics of insulin observed by circular dichroism spectra. The mechanism of insulin oxidation processes on unmodified SPGEs and nanoparticle-modified screen printed electrodes is discussed, along with the question of which and how many amino acids are subject to the insulin oxidation process. Scanning electron microscopy was used to characterize the electrodes after the modification with nanostructures; small features were visible after the deposition of bismuth film, increasing the electrode surface.

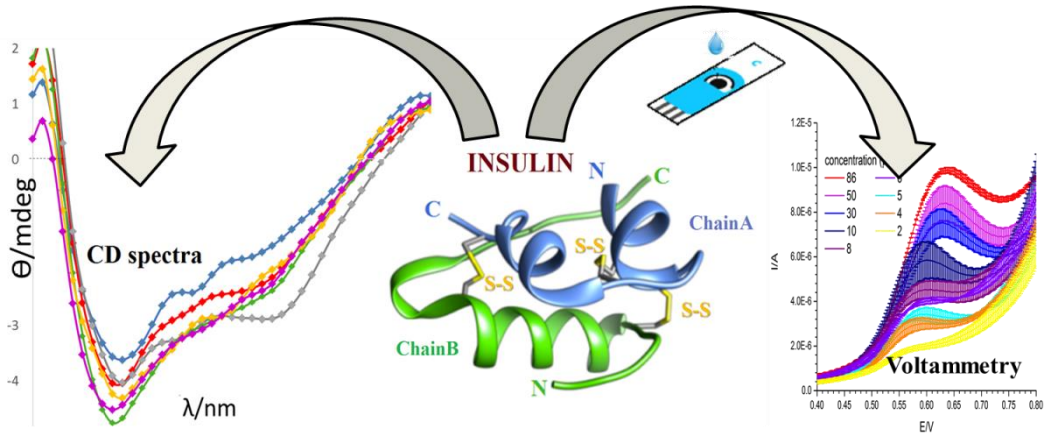

Figure: Graphical representation of both approaches of the study of human insulin: spectroscopic and electrochemical experiments. The structure of insulin consists of two chains connected by disulfide bridges. The left picture shows the CD spectra of insulin in buffered solutions of different pH. The right picture illustrates the oxidative voltammetric signals of insulin on SPGE as a function of its concentration.

A detailed understanding of the synergetic function of nanostructured surfaces in the kinetics of insulin oxidation may be the starting point for the preparation of screen-printed electrode microarray assembly, which will guarantee fast insulin analysis, high throughput, and low

sample consumption. Thus, the results of our study can be helpful for the development of non-enzymatic sensitive insulin sensors as well as for biochemical research of insulin in medicine and pharmacology.

ACKNOWLEDGMENT

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PRESENTATIONS OF COMPANIES

APPLICATION OF MULTICHANNEL ELECTROCHEMICAL DETECTION WITH COULOMETRIC EFFECTION IN THE GRADIENT HIGH PERFORMANCE LIQUID CHROMATOGRAPHY AND IN THE FLOW INJECTION ANALYSIS TO ANTIOXIDANT EVALUATION

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Plant materials are highly complex biological matrices and contain many diverse phytochemicals, relatively few of the analytes are electrochemically active. Natural antioxidants have beneficial effects on human health. There is a big interest in techniques for the determination of natural antioxidants. Multi-channel electrochemical detector CoulArray (ESA Inc., Chemlsford, MA, USA) is only one to be compatible with the gradient elution mode /1/ providing separation complex mixtures of extractable electrochemically active compounds and their identification according hydrodynamic voltammogram and /or predomint, dominant and postdominat peak ratio.

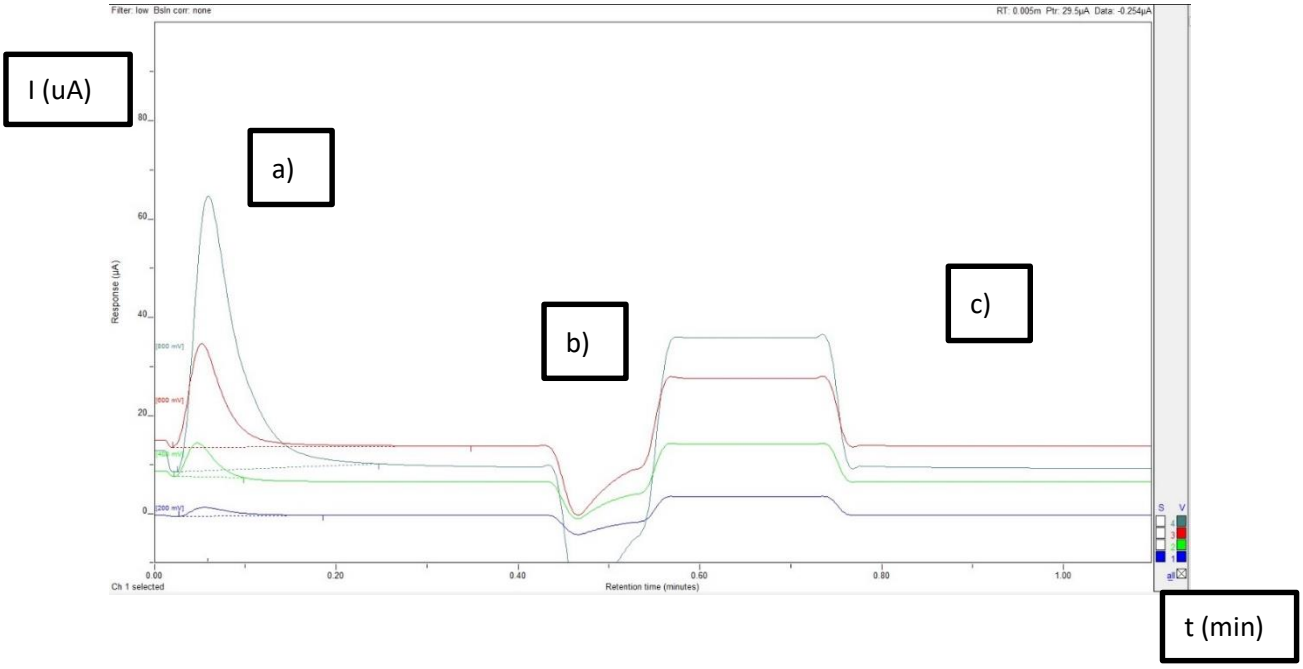

Figure: FIA / CoulArray analysis with working potentials 200, 400, 600 and 800 mV

There is on (Fig. 1) a record of the FIA / CoulArray analysis with applied working potentials of 200, 400, 600 and 800 mV with automatic peak integration, electrochemical cleaning and autozero within 66 s. Time period a) shows the responses on the working electrodes of 200, 400, 600 and 800 mV potentials. The area under the response curve corresponds to the charge transferred between the electroactive analyte and the working porous graphite electrode. Time

period b) shows the electrochemical cleaning of the working electrodes by applied on the working electrode first a negative potential (-800 mV) and then a positive potential (+1000 mV). In the time period autozero c) a signal is set to zero baseline.
Electrochemical cleaning is the most important step enabling the use multichannel electrochemical porous graphite sensors in the flow injection analysis of polyphenolic natural monomers, dimers, tetramers, oligomers and polymers. The electrochemical detection employed in antioxidant analysis is based on oxidation reaction associated with production in mobile phase insoluble chemical species of various chemical structure. These probably higher molecular compounds are hidden for general chromatographer who uses standard chromatography methodologies to separate and identify chemical compounds working with commercially available standards. The flow injection analysis is able to collect charge of these hidden unknow chemicals providing better information about the antioxidant power of the whole portfolio extractable electroactive compounds.

ACKNOWLEDGEMENT

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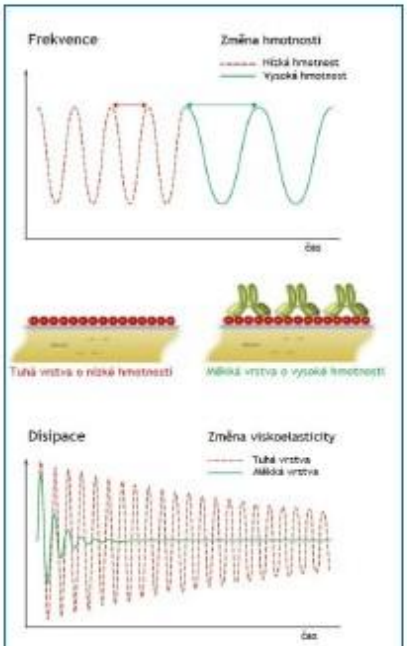
Q-Sense
[Together with Biolin Scientific]

METODA QCM-D
MĚŘENÍ HMOTNOSTI, TLOUŠŤKY A STRUKTURNÍCH VLASTNOSTÍ MOLEKULÁRNÍCH VRSTEV



Metoda QCM

Základem měření QCM je AT-štěpený křemenný krystal, připojený ke zdroji střídavého napětí pomocí dvou elektrod a pokrytý tenkou vrstvou kovu. Adsorbce molekul na povrch krystalu vyvolá změnu rezonanční frekvence, na které krystal kmitá. Z této změny lze vypočíst změnu hmotnosti. Jedná se o velmi citlivou metodu umožňující sledovat rozdíly hmotností v řádu nanogramů, interakce molekul a kinetiku probíhajících reakcí a to v reálném čase a bez nutnosti značení.

QCM-D

Patentovaná technologie stanovení disipace neboli ztráty energie umožňuje měřit rychlost, kterou vrstva látky na povrchu krystalu pohlcuje energii. Je zaznamenána poté, co je krátce přerušeno buzení křemenného krystalu střídavým proudem, probíhající v krátkých intervalech v průběhu celého měření. Jako výsledek dostaneme spolu s křivkou změny rezonanční frekvence také křivku disipace energie. Ta vypovídá o viskoelastických vlastnostech adsorbované vrstvy.

APLIKACE:

- Adsorpční a desorpční kinetika
- Příprava a uchování léčiv
- Solární články nové generace
- Imunokompatibilita implantátů
- Polyelektrolytické vrstvy
- Interakce protein-DNA
- Tvorba lipidových vrstev
- Koroze palivových článků
- Strukturní změny proteinů
- Účinnost detergentů
- Aktivita enzymů
- Interakce Ab-Ag
- Degradace celulózy
- Nanotoxikologie
- Analýza toxinů
- Buněčná adheze
- DNA hybridizace
- Pokovování

Q-Sense E4 Auto

- 4 paralelní automatická měření
- Moduly: elektrochemický, elipsometrický, mikroskopický, atd.

Q-Sense Omega Auto

- 2 x 4 paralelní automatická měření
- Zabudovaný termostat
- Mikrotitrační destička s autosamplerem

CHROMSPEC SPOL. S R.O.
Mníšek pod Brdy
Brno
Tel.: 318 599 083
Tel.: 547 246 683
info@chromspec.cz
www.chromspec.cz

Zařízení pro přesný ohřev a chlazení

REGULACE NAPROGRAMOVANÉ TEPLOTY S PŘESNOSTÍ 0,1°C, VYSOKÁ VARIABILITA: OBJEMY, PROVEDENÍ (NEREZ, HLINÍK, PLAST) A ROZSAH TEPLOT

Skříňové termostaty ST, Q-Cell

objem od 70 l do 1460 l

- teplotní rozsah: +3°C až +40°C/ +70°C
- vynucená cirkulace vzduchu, měření teploty v reálném čase
- alarmy překročení nastavené teploty, výpadku napětí, …

Laboratorní chladničky CHL, Q-Cell-CHL

objem od 70 l do 1460 l

- teplotní rozsah: 0°C až +15°C
- nízkoteplotní verze: -10°C až +10°C
- vynucená cirkulace vzduchu

Laboratorní mrazničky

Skříňové modely nabízejí objem truhlicové mrazničky **objem od**

- teplotní rozsah: -25/- 40°C až
- vynucená nebo přirozená

ZLN,ZLW, Q-Cell

od 85 - 447 litrů
200 – 600 litrů.

0°C
cirkulace vzduchu

Sterilizátory SR a Sušárny SL, QSL-1

objem od 15 l (SL), 56 l (SR) do 1005 l

- teplotní rozsah: od 5°C nad teplotou pracovního prostředí do +250°C sterilizátory / do +300°C sušárny
- vynucená nebo přirozená cirkulace vzduchu, přednastavené programy sterilizace, automatické blokování dveří…

Klimatické komory KK

objem od 115 l do 1485 l

- teplotní rozsah -10°C. + 60 °C
- rozsah vlhkosti: 30 - 90%
- možnost programování a regulace teploty, vlhkosti a intenzity osvětlení,
- FIT systém – nastavení osvětlení den/noc
- možnost volby barvy světla (LED) panel

NOVINKA: Přenosný coulometrický analyzátor PCA2

Analýzy těžkých kovů, aniontů, ionogenních organických látek, rozpuštěného O_2, SO_2...

- řízen vlastním mikroprocesorem.
- vybaven napájecí baterií pro měření v terénu nebo ve výrobním provozu
- meze stanovitelnosti od 10 μg/l, lineární rozsah koncentrací až 4 řády

Odběr a úprava vzorků

Nástroje pro odběr plynných, kapalných i pevných vzorků ve výrobě, kontrole, životním prostředí

Více než 40 druhů nástrojů, např.:

- vakuová pumpička pro odběr kapalin ze sudů, cisteren apod. bez kontaminace vzorkovacího nástroje, i pro odběr hořlavin do předem evakuovaných lahví
- ventil pro kontinuální odběr vzorků paliv z cisteren
- nástroje pro odběr kapalin z velkých hloubek
- dvouplášťový vzorkovač pro současný odběr několika vzorků sypkých materiálů

Nástroje pro drcení, mletí, tavení a zmenšování vzorků

- čelisťové drtiče, laboratorní mlýnky, lisy
- rotační děliče pro definované rozdělení sypkých vzorků
- elektrické tavičky pro přípravu vzorků k RTG analýze

Dále nabízíme:

Laboratorní přístroje

- plamenové fotometry, spektrofotometry
- gelová elektroforéza, osmometry, kryometry
- pH-metry, ionometry, konduktometry, refraktometry – laboratorní i přenosné

Příslušenství a spotřební materiály

- elektrody pro měření pH, ISE, konduktometrii…
- pro AAS - výbojky, grafitové kyvety, ICP - hořáky, zmlžovače…

Laboratorní potřeby a zařízení, laboratorní nábytek

- homogenizátory, ultrazvukové lázně, laboratorní váhy a termogravimetry
- počítačky kolonií bakterií
- celoplastové digestoře, skříně na kyseliny...

Certifikované referenční materiály

- čisté látky, jednoprvkové a víceprvkové roztoky pro kalibraci
- matriční – půdy, horniny, nerostné suroviny, vody, potraviny, rostliny, tkáně, kovy, cement...

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Book of abstracts

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