47th Heyrovsky Discussion

on

Electrochemistry of Organic and Bioactive Compounds

Třešť, Czech Republic

May 25th-29th, 2014

BOOK OF ABSTRACTS


IBP


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J. HEYROVSKY INSTITUTE OF PHYSICAL CHEMISTRY OF THE AS CR, V.V.I., PRAGUE,

INSTITUTE OF BIOPHYSICS OF THE AS CR, V.V.I., BRNO,

AND

CENTRAL EUROPEAN INSTITUTE OF TECHNOLOGY, MASARYK UNIVERSITY, BRNO

BOOK OF ABSTRACTS

47th Heyrovsky Discussion

on

Electrochemistry of Organic and Bioactive Compounds

On the occasion of the 55th anniversary of awarding
Professor Heyrovsky by Nobel Prize for the invention of polarography

Editors:

Tomáš Navrátil, Miroslav Fojta, Martina Fojtová and Ivana Mužíková

Třešť Czech Republic

May 25th-29th, 2014

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MAIN THEME AND PROPOSED DISCUSSION TOPICS:

Electrochemistry and electroanalysis of drugs, bioactive compounds, genotoxic and ecotoxic substances

- *novel electrochemical biosensors and their applications*
- *application of new electrode materials*
- *combination of electrochemistry with other techniques (e.g., spectral methods)*

Structure-reactivity relationship in redox-active molecular systems

- *redox mechanistic studies of organic and coordination compounds*
- *bond activation by electron transfer*
- *correlation of experimental data with theoretical calculations and other related problems*
- *electron transfer in molecules with multiple redox centers*

Biopolymer electrochemistry

- *electrochemical properties and analytical use of natural and synthetic nucleic acids and redox-labelled nucleic acid conjugates*
- *protein electrochemistry and electroanalysis: structure and interaction effects*
- *electrochemistry of natural and chemically modified carbohydrates*

Biological membranes, their mimics and transporting processes

- *transporting processes across membranes*
- *transport through nanopores*

Novel materials and nanotechnology for electrochemical sensors

- *electrode materials and surface modifications*
- *nanostructured surfaces*
- *nanoobject for biopolymer labeling*

Program

Sunday, May 25	
After-noon:	**Transportation to Trest** (time of bus departure from the IPC JH will be specified)
cca 16:30	**Registration** (registration desk will be open through the conference)
19:30	***Get-together Party***

Monday, May 26			
8:45	9:00	***Opening of the conference***	
9:00	9:30	Ferapontova	Chair: **Flechsig**
9:30	10:00	Hocek	
10:00	10:15	Havran	
10:15	10:40	***Coffee Break***	
10:40	11:10	Labuda	Chair: **Paleček**
11:10	11:30	Sato	
11:30	11:45	Campos	
11:45	12:00	Kékedy-Nagy	
12:00	12:15	Špaček	
12:15	14:00	***Lunch***	
14:00	14:30	Bowater	Chair: **Ferapontova**
14:30	14:50	Fojta	
14:50	15:10	Paleček	
15:10	15:30	Takenaka	
15:30	15:50	***Coffee Break***	
15:50	16:20	Flechsig	Chair: **Labuda**
16:20	16:40	Gál	
16:40	16:55	Enache	
16:55	17:15	Barath	
17:45		***Dinner***	
19:00		***Concert (Trest Castle)***	

Tuesday, May 27			
9:00	9:30	Economou	Chair: **Gál**
9:30	9:50	Ostatná	
9:50	10:05	Černocká	
10:05	10:20	Vargová	
10:20	10:40	***Coffee Break***	
10:40	11:00	Kolivoška	Chair: **Economou**
11:00	11:15	Lopes	
11:15	11:35	Opršal	
11:35	11:50	Drbohlavová	
11:50	12:05	Majzlíková	
12:05	12:20	Kynclová	
12:20	13:45	***Lunch***	
14:00	17:30	***Excursion (Jihlava)***	
18:00		***Dinner***	
20:00		***Open Air Beer & Sausage Party***	

Wednesday, May 28			
9:00	9:30	Kutner	Chair: **Ariño**
9:30	9:50	Dazie	
9:50	10:10	Kantnerová	
10:10	10:30	***Coffee Break***	
10:30	10:50	Zyatdinova	Chair: **Kutner**
10:50	11:10	Mendkovich	
11:10	11:30	Mikysek	
11:30	11:50	Kocábová	
11:50	12:10	Lachmanová	
12:10	14:00	***Lunch***	
14:00	14:30	Ariño	Chair: **Zyiatdinova**
14:30	14:50	Šestáková	
14:50	15:10	Navrátil	
15:10	15:30	Le	
15:30	15:50	***Coffee Break***	
15:50	16:05	Zámečníková	Chair: **Mikysek**
16:05	16:20	Mansfeldová	
16:20	16:35	Tiribilli	
16:35	16:50	Šelešovská	
16:50	17:05	Ramešová	
17:05	17:20	Langmaier	
17:20	17:35	Nováková	
18:00		***Farewell party***	

Thursday, May 29
Bus transportation to Prague (after breakfast, departure time will be specified

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Monday, May 26th, 2014

Electric Field Effects on Interfacial Behaviour of Surface-Tethered DNA

Elena Ferapontova

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Electron transfer (ET) in biological systems depends to a large extent on ET pathways, which can be different from the naturally expected ones when ET proceeds under conditions of the electrode reaction [1, 2]. That is a particular case of DNA molecules tethered to electrodes, either labelled with redox probes or interacting with redox indicators [1, 3-5].

With surface-tethered DNA the electrochemical signal from the redox molecule interacting with/bound to the DNA duplex was shown to not obligatory result from the ET mediated by the π-stacked DNA duplex but from the alternative mechanisms of ET and essentially depend on the whole structural design of the DNA-electrode systems and modes of interaction between the DNA molecule and the redox probe [3-7].

Here, we discuss the effect of the electric double layer (EDL) structure on the mechanism and kinetics of ET between the DNA-bound redox probe and the electrode for the case of loosely packed DNA monolayers and the negatively charged electrode surface, providing the up-right orientation of the DNA duplex at the electrodes [8,9]. The cases of the alkanethiol linkage and direct chemisorption of the DNA to the electrodes through the modified DNA bases will be discussed [8]. Modelling of the EDL effects on the kinetics of ET provided further understanding of the effect of electric field on the complex interfacial behaviour of the DNA duplexes tethered to the electrodes [9].

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Polymerase Synthesis of Base-Modified DNA. From Redox Labelling to Chemical Biology

Michal Hocek [1,2]

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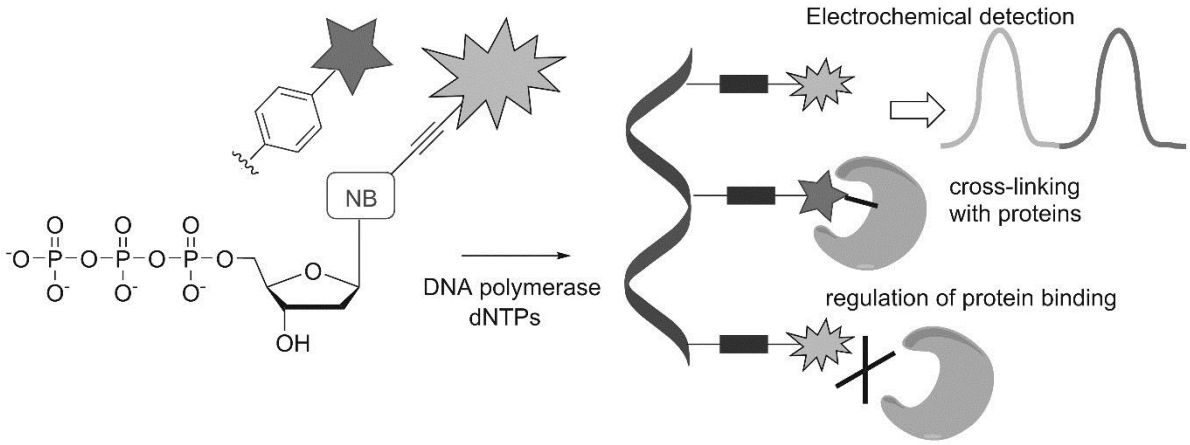
An efficient two-step methodology of construction of functionalized nucleic acids was developed by a chemo-enzymatic approach using aqueous-phase cross-coupling reactions of nucleotides followed by incorporation by DNA polymerase [1]. The methods are applied in the synthesis of redox-labelled oligonucleotide or DNA probes for electrochemical detection and applications in diagnostics [2, 3], as well as in the synthesis of modified DNA for applications in chemical biology (regulation of protein binding or bioconjugations) [4-7].



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Acknowledgments

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7-Deazapurines a New Targets for Redox Active DNA Labelling

Luděk Havran, Jan Špaček, and Miroslav Fojta

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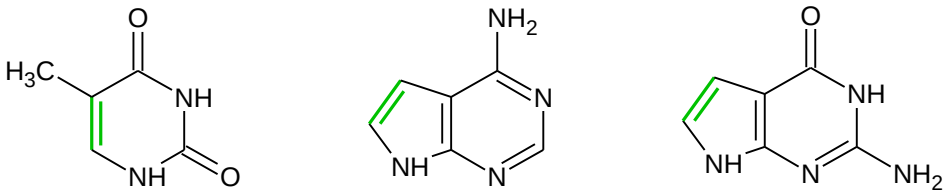
Natural DNA electroactivity has found a wide use in electrochemical analysis of the DNA interactions and damage [1]. For some applications including development of DNA hybridization sensors it is advantageous to apply redox active tag(s) to improve specificity of the analysis. Complexes of osmium tetroxide with nitrogen ligands (Os,L), which produce with DNA stable covalent adducts, are well established useful electroactive DNA labels [2]. If 2,2'-bipyridine (bipy) is used as ligand, Os,bipy selectively react with thymine residues in single-stranded DNA. Corresponding adducts give at mercury and carbon electrodes a set of voltammetric signals due to reduction/oxidation of the central Os atom. Moreover the final reduction step at the hanging mercury drop electrode (HMDE) is coupled to catalytic hydrogen evolution allowing determination of low concentrations of the osmium-labeled DNA or rare adducts in large excesses of unmodified DNA. Using basal-plane pyrolytic graphite electrodes (PGE) allows a direct analysis of reaction mixtures in combination with extraction of unbound Os,L from electrode surface by organic solvent [3].
7-deaza analogues of purine nucleobases contains the same structural motive (C7=C8 double bond) reactive to Os,L as the C5=C6 bond in the pyrimidine nucleobases (Fig. 1). Therefore a similar reactivity of these nucleobases to Os,L can be expected. In this contribution we will present for the first time electrochemical analysis of synthetic oligonucleotides containing 7-deaza adenine or 7-deaza guanine adducts with Os,bipy.



T 7-deazaA 7 deazaG

Fig. 1: Structures of nucleobases with assumed reaction sites.

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Acknowledgments

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DNA Biosensors with Protective Outer-Sphere Membranes

Ján Labuda, Lenka Hlavatá, Viktor Gajdoš, and Lucia Šteffelová

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Due to surface fouling and other interactions, physical stability and response of chemical sensors used in analytical samples of complex matrices are generally affected by the presence of high molecular weight and surface active compounds. The same is true for DNA-based biosensors known as effective tools and warning devices in tests of DNA association interactions with low molecular weight compounds like drugs and potentially toxic chemicals as well as tests of DNA integrity in the presence of various chemical and physical agents [1, 2]. To achieve necessary selectivity and eliminate interferences effects, outer-sphere protective membranes are typically used at the construction of biosensors [3, 4].
Here we report on properties and application of the DNA biosensors with carbon electrode transducers and polymer membranes. Nafion, chitosan, polyvinyl alcohol and other polymers deposited on the DNA biorecognition layer have been tested with respect to value and stability of the biosensor response after its previous incubation in matrices of beverages like fruit juices, coffee, beer and vines. A complex detection approach based on combination of several modes such as the guanine moiety SWV anodic response, CV response of the hexacyanoferrate anion as a redox active dsDNA indicator and charge transfer resistance obtained by electrochemical impedance spectroscopy has been utilized to evaluate antioxidative properties of beverages regarding the oxidative DNA damage [5, 6].

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Acknowledgments

This work was supported by the Scientific Grant Agency VEGA of the Slovak Republic (Project No. 1/0361/14) and the Competence Center for SMART Technologies for Electronics and Informatics Systems and Services, ITMS 26240220072, funded by the Research&Development Operational Programme from the ERDF.

Development of Electrochemical Telomerase Assay Using Ferrocenyl Naphthalene Diimide Derivatives

Shinobu Sato [1,2], Yuki Hori [1], Mana Hayakawa [3], Masaaki Kodama [3,5], Tatsuji Nishihara [4, 5], Kazuhiro Tomonaga [3,5], and Shigeori Takenaka [1,2]

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Telomerase is expected to serve as a new tumor marker and its activity has been detected by Telomerase Repeat Amplification Protocol (TRAP), which contains some laborious manipulations such as PCR and gel electrophoresis [1].
We have been developing an electrochemical gene detection method with ferrocenylnaphthalene diimide (FND) as an electrochemical hybridization indicator [2]. The advantages of this method lie in the speed and high sensitivity. In fact, it is possible to detect telomerase activity electrochemically without PCR [3]. Where telomerase activity is present in a sample solution, a telomerase substrate (TS)-primer immobilized on the electrode is elongated to yield a telomeric repeat sequence and the resulting products can form a tetraplex DNA. FND binds to the tetraplex formed on the electrode to give rise to an electrochemical signal whose magnitude reflects telomerase activity. Herein, we tested telomerase activity in saliva, oral epithelial cells, and solid tumor for diagnosis of tongue cancer [3].
On the basis of this difference individual clinical samples were judged telomerase positive, ambiguous or negative. The positive rate in the cancerous tissue and exfoliated cells of the patients was 85 and 80%, respectively, whereas the corresponding values were 50 and 10% by the Telomerase Repeat Amplification Protocol as an existing method. Furthermore, the positive rate amounted to 100% in early tumors smaller than 2 cm by ECTA. Likewise, 95 and 80% of biopsy and exfoliated cells of healthy individuals were judged negative properly. The electrochemical method yielded high hit rates for cancerous and normal cells, especially in exfoliated cells, making this low invasive test suitable for oral cancer diagnosis.

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DNA Biosensor Exploiting Direct DNA Immobilization onto Gold via Phosphorothioated dA Tags

Rui Campos [1], Alexander Kotlyar [2], and Elena E. Ferapontova [1]

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Single nucleotide polymorphism (SNP) can be detected using DNA hairpin beacons [1], reaction with methylene blue (MB) [2] or by analysis of the signal of MB covalently attached to DNA [3]. Usually DNA is modified with an alkanethiol linker at the 3' or 5' end [4], and then self-assembled on the Au surface. Using alkanethiol linkers has some drawbacks: the necessity of chemicals to break the S–S bond (with tris(2-carboxyethyl)phosphine); the length of the alkane chain may affect the DNA signal [5], and, the most important, alkanethiol linkers are normally synthetically introduced, restricting the length of the modified DNAs. Here, we report self-assembly of DNA, modified with a phosphorothioated dA (dA*) tag that can be easily introduced by molecular biology approaches, onto gold electrodes as a new method of DNA immobilization for electrochemical studies.

The rate of electron transfer (ET) between the electrode and methylene blue intercalated in DNA immobilized either via the traditional alkanethiol linker (C6) [6] or the dA* tag was analyzed at low surface coverages [7] (< 3 pmol cm^{-2}). Higher ET rates, better stability and sensitivity for SNP were observed for DNA assembled via the dA* tag. The SNP discrimination supported the DNA mediated mechanism of ET. The results allow the development of genosensors based on longer DNA sequences than those synthetically available: the removal of the synthetic linker can allow DNA-mediated ET electrochemical studies with genomic DNA or novel design of aptasensors [8], with improved characteristics compared to those achieved with alkanethiol linkers.

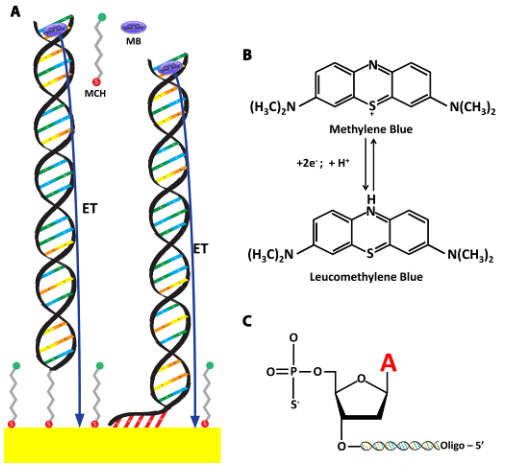

Figure. (A) Schematic representation of the gold electrode modification with alkanethiol- and dA*-modified double stranded (ds) DNA and DNA-mediated ET in the duplex, (B) Redox reaction of methylene blue at pH higher than 6, and (C) Chemical structure of phosphorothioated adenosine.

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Electroanalysis of Modes of Methylene Blue Binding to Gold-Tethered DNA

László Kékedy-Nagy, Rui Campos, and Elena Ferapontova

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Electron transfer (ET) in biological systems depends to a large extent on the ET pathways predetermined by environmental interactions of redox active species with biomolecules, particularly important in the case of double-stranded (ds) DNA and redox probes either capable of intercalation into the DNA duplex or not [1-3]. Here, interactions between dsDNA tethered to gold electrodes through the alkanethiol linker and the positively charged Methylene Blue (MB) redox indicator capable of intercalating, groove and electrostatic binding to dsDNA were studied at different ionic strength and MB concentrations [4]. Modes of MB interactions with dsDNA were shown to be sequence-specific and dependent on the concentration of MB in solution, with MB electrochemistry changing from diffusion-limited to the one whose kinetics is limited by surface-confined ET with the increasing concentration of MB and decreasing ionic strength. The ET kinetics are discussed within the context of different modes of interactions of MB with dsDNA are restricted to the minor and major groove binding and specific conditions of MB intercalation providing DNA-mediated ET are specified. The shown different modes of MB-DNA interactions are particularly important for understanding the ET properties of DNA and for the development of new concepts of biosensors based on DNA-mediated ET reactions.

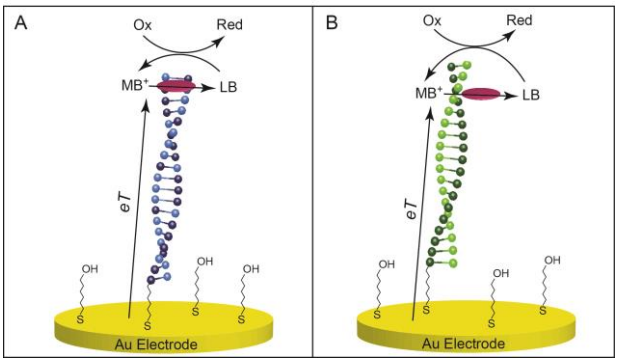

Fig.1 :

*Figure from the PhD thesis of Alireza Abi

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Oxidation of Long DNA Homopolymer Tails on Graphite Electrodes

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Previously we showed that ratiometry can be used for DNA sequence analysis [1]. Here we present a ratiometric study of products of terminal transferase (TdT) reaction [2] based on oxidation of G and A on the pyrolytic graphite electrode [3]. The method is based on a rule that area of a redox signal corresponds to amount of the studied substance adsorbed on the electrode surface. By using ratiometry we can disregard variations of the electrode surface area and variations in total amount of DNA absorbed on the surface of the electrode to determine simultaneously relative amounts of two or more electroactive species. We showed that this method can be reliably used for analysis of synthetic oligonucleotides tailed with poly(dA) tail up to hundreds of dA (Fig. 1), and to determine average fragment lengths of cleaved genome DNA elongated with controlled length poly(dA) tails.

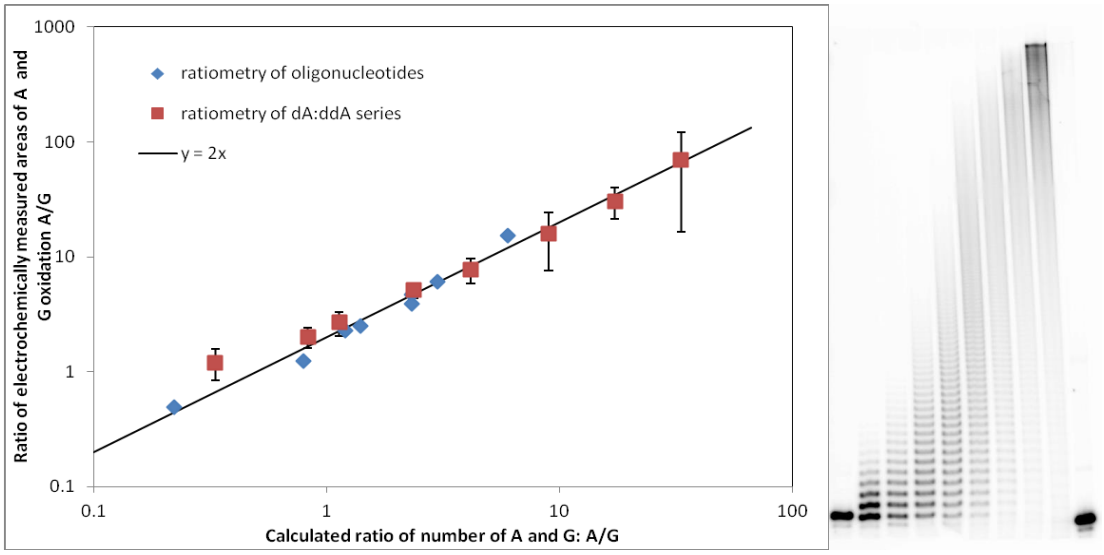

Fig. 1: Comparison of electrochemically measured oxidation signals of A and G to a total amount of A and G in the studied molecules. 9 oligonucleotides with A/G ratios varying from 0 to 6 were used. For series of dA:ddA samples, terminal transferase was used to prepare different tail lengths using different dA:ddA ratio. Each sample had a tail with $(dA)_n$ddA, where *n* was a distributive value. Average value of *n* in different samples was from 0 to 100 (as shown on polyacrylamide gel electrophoresis autoradiogram on the right).

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Acknowledgments

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Biophysical Chemistry Studies of Protein-Nucleic Acid Interactions

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Nucleic acids are long polymers of nucleotides that are central to all life. In the form of deoxyribonucleic acid (DNA) they provide the genetic sequence that encodes hereditary information, and as ribonucleic acid (RNA) they decode and express the information to allow all cellular processes to take place. In order for their information to be made available to cells, proteins must interact with the nucleic acids, ensuring that the molecules are replicated, transcribed and translated as required. Due to the significance of such protein-nucleic acid interactions to cellular metabolism they have been widely studied [1, 2]. Some proteins interact with the sequence information - the bases - in nucleic acids, which allows formation of specific complexes with relatively high affinity between the different molecules. By contrast, other proteins interact with the structure adopted by the nucleic acid; since a vast array of nucleic acid structures can form, the strength and specificity of these types of interactions is highly variable and, in some cases, they can be relatively weak and non-specific. Biophysical chemistry methodologies have been particularly useful for characterising factors that influence these wide range of interactions [3].
This presentation will provide an overview of the range of biophysical chemistry methods that have been used to study protein-nucleic acid interactions. Each experimental approach has advantages and disadvantages, meaning that a range of techniques must often be used to fully characterise a complex. Data will be presented for studies of well-characterised DNA repair proteins interacting with DNA molecules that have non-standard types of structure. The combination of molecular biology and biophysical chemistry methods has produced flexible assays, which show that pH and ionic strength influence the binding of the proteins to a variety of DNA structures and sequences [4]. As will be demonstrated for a range of proteins involved in nucleic acid metabolism, the inclusion of fluorescently-labelled nucleic acids in such combined approaches allows for detailed biochemical and biophysical characterisation of macromolecular complexes.

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Electrochemical methods for the detection of DNA-protein interactions

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DNA-protein interactions are of critical importance for controlling vital cellular functions, such as chromatin modelling and remodelling processes, DNA replication, transcription and repair, etc. To study DNA protein interactions in vitro, a number of experimental techniques which utilize various detection principles have been applied. Electrochemical biosensors and bioassays belong to promising tools for detecting biomolecular interactions and/or their impacts on DNA or protein structure. This presentation will provide several examples of electrochemical methods applied in this field.

Affinity interactions on a solid support represent the basis of a number of well-established biochemical assays, such as ELISA, or biosensors employing e.g., surface plasmon resonance or piezoelectric detection. Similarly, electrochemical biosensor for DNA-protein binding can be created via immobilization of one of the interacting molecule (e.g. DNA) on an electrode surface. This probe (receptor, in specific cases aptamer [1]) interacts with the other molecule in solution, forming a complex the presence of which can be detected by different, label free or indicator-based techniques. An alternative approach consists in separation of the interaction and detection steps on two different surfaces [2]. Typically, magnetic beads modified with immobilized DNA or the protein can be utilized for an efficient capture of the given interacting partner via the affinity interaction. After this separation/enrichment step, the captured molecules can be detached from the beads surface and determined electrochemically using intrinsic electrochemical activity of the DNA or protein [3], or using electrochemical signals of an electroactive label bound to a DNA probe.

Binding of a protein to DNA can cause a change in the DNA structure, such as unwinding or bending of the DNA duplex, or flipping-out of certain nucleobase residues. It has been proposed that these phenomena are connected with disruption of base pair stacks in the DNA double helix, with concomitant diminution of DNA-mediated charge transfer. Sensor devices working on this principle have been designed [4]. A specific case of DNA structure alteration due to interacting with a protein is action of DNA modifying enzymes. DNA nicking [5] or ligation [6] activities have been detected by electrochemical methods sensitive to formation (sealing) of DNA strand breaks. Activity of other enzymes, such as polymerases, can easily be assayed electrochemically via monitoring of synthesis of DNA stretches tagged by natural or modified nucleobases.

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Sensing of Tumor Suppressor Protein p53-DNA Complex at an Electrified Interface

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Electrochemical biosensors have the unique ability to convert biological events directly into electrical signals suitable for parallel analysis. Here we utilize specific properties of constant current chronopotentiometric stripping (CPS) in the analysis of protein and DNA-protein complex layers [1]. Rapid potential changes at high negative current intensities (I_{str}) in CPS are utilized in the analysis of DNA-protein interactions at thiol-modified mercury electrodes [2-4]. P53 core domain (p53CD) sequence-specific binding to DNA results in a striking decrease in the electrocatalytic signal of free p53. This decrease is related to changes in the accessibility of the electroactive amino acid residues [2], in the p53CD-DNA complex. By adjusting I_{str} and temperature, weaker non-specific binding can be eliminated or distinguished from the sequence-specific binding (Fig. 1). The method also reflects differences in the stabilities of different sequence-specific complexes, including those containing spacers between half-sites of the DNA consensus sequence. The high resolving power of this method is based on the disintegration of the p53CD-DNA complex by the electric field effects at a negatively charged surface and fine adjustment of the millisecond time intervals for which the complex is exposed to these effects. Picomole amounts of p53 proteins and DNA were used for the analysis at full electrode coverage but we show that even 10-20-fold smaller amounts can be analyzed. Our method cannot however take advantage of very low detection limits of the protein CPS detection because low *Istr* intensities are deleterious to the p53CD-DNA complex stability at the electrode surface. These data highlight the utility of developing biosensors offering novel approaches for studying real-time macromolecular protein dynamics.

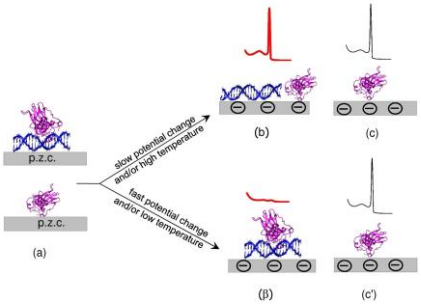

Fig. 1

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Detection of Nucleic Acid or its Related Enzyme Based on Ferrocenyl Ligands

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Electrochemical DNA detection has been the focus of many studies from a standpoint of health-care biochips and other related fields. Along this line, we have been developing an electrochemical DNA detection technique based on ferrocenylnaphthalene diimide (FND) [1]. Since naphthalene diimide derivatives are known to bind to double stranded DNA (dsDNA) with threading intercalation, they are stabilized by pseudo-catenane formation. For example, ferrocenylnaphthalene diimide (FND) can be efficiently concentrated on dsDNA, thus enabling its electrochemical detection. Since dsDNA is formed between single stranded target DNA and DNA probe, the target DNA can be detected electrochemically, using DNA probe-immobilized on the electrode. Further stabilization of naphthalene diimide derivatives as a complex with dsDNA on the electrode is expected to lead to more precise and selective detection of target DNA. This approach was realized by the formation of inclusion complexes of ferrocene with β-cyclodextrin (β-CD) for the FND - dsDNA complexes on the electrode [2]. Combination of adamantylnaphthalene diimide (AND) and ferrocenyl-β-CD (Fc-CD) gave new supramolecular DNA detection assays coupled with DNA probe-immobilized electrodes [3]. The electrochemical signal increased only upon formation of the Fc-CD/AND complex bound to dsDNA on the electrode. Naphthalene diimide carrying ferrocene and β-CD realized "signal on" type detection of dsDNA in homogenous solution [4]. Redox peak of ferrocenylnaphthalene diimide shifted positively due to the formation of its complex with β-cyclodextrin as described above. When this complex can collapse upon the addition of double-stranded DNA, its redox potential shifted negatively and can be applied for the homogenous detection. According to this idea, polymerase chain reaction (PCR) product from *Porphyromonas gingivalis*, which is important for the diagnosis of periodontal disease, was quantitatively detected with high sensitivity [5]. We also evaluated telomerase activity in the lysate of tumor tissue and surrounding cells of oral cancer patients by an electrochemical technique, dubbed the electrochemical telomerase assay (ECTA) [6]. On the other hand, ferrocenyl oligonucleotide-immobilized electrode was used to detect its related enzyme such as DNase I or RNase A and we successfully detected these enzyme activities using the current response of the sensor electrode decreased with increasing enzyme concentration [7,8].

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New Materials and Nanostructures for Heated Electrochemical Sensors

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Directly heated gold wire electrodes were galvanically modified with a layer of gold nanostructures. This template-free method had been applied before with gold disk electrodes and was tested for DNA hybridization detection. We found an increase by factor 9 for signal-to-noise-ratio coupled with broader linear range and improved thermal regeneration of the DNA probe layers [1]. Nanostructured electrode surfaces are therefore very useful for microelectrode arrays because they increase greatly the electro-active surface area, making signals in the nA to pA range less prone to electromagnetic noise.
Gold nanostructures on a microwire electrode (Fig. 1) seemingly affect the microelectrode behaviour producing peak-shaped signals in cyclic voltammetry. Sigmoidal voltammograms can be regained at elevated electrode temperature [2]. On the other hand, considering the gold oxide reduction peak, CVs in 0.5 M H_2SO_4 reveal that the increase in electroactive surface area is very comparable to the earlier findings. Various aspects of mass transport at this new type of working electrode will be discussed in this contribution.
Another new modification of heated gold wire electrodes addresses the negative potential range [3]. Galvanic bismuth film deposition on gold micro-wires leads to increased negative potential range. The Bi film can be stabilised by glowing in an argon atmosphere forming gold bismuth alloys. Determination of picric acid could be improved this way.

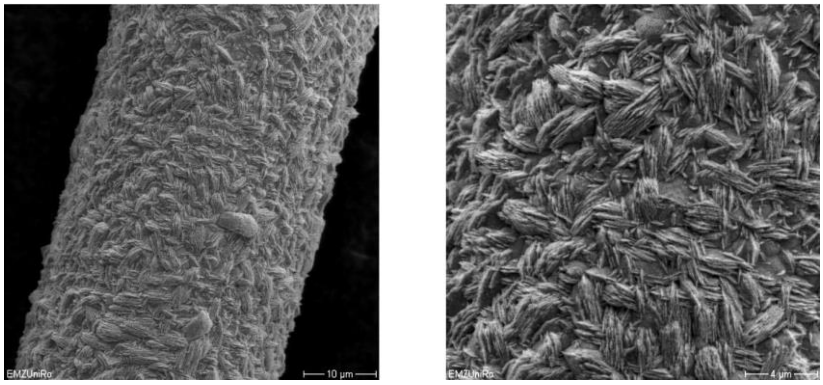

Fig. 1: Nanostructured surface of a heated gold microwire.

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Characterization of Catalytic Properties of Human Eneteropeptidase by Electrochemical Methods

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Protease, known for its application in cleavage of fusion partners in molecular biology is Enterokinase. Enterokinase (EC 3.4.21.9) is a serine protease produced found in the intestinal brush border membrane of the duodenum, which activates trypsinogen by the cleavage of the N-terminal peptide, followed by the conserved sequence of four aspartic acids and one lysine. This is the exact sequence of five amino acids highly specifically recognized by Enteropeptidase, which cleaves N-terminal part from C-terminal, immediately after these amino acids. Its precursor is a single chain polypeptide composed of heavy (82-140 kDa) and light (35-82 kDa) chain. Enterokinase's high specificity rate makes it the enzyme of choice for cleavage of fusion proteins produced in bacteria.

In general, UV-Vis and/or fluorescence spectroscopy are the methods of choice in the characterization of basic biochemical properties of enzymes, such as maximum reaction rate (V_{max}), Michaelis constant (K_M) and turnover number (k_{cat}). However, in the case of some type of substrates (e.g. fusion proteins) no characteristic UV-Vis or fluorescent spectrum is obtained. Therefore, electrochemical methods might be helpful for biochemical enzyme characterization. To prove the suitability of electrochemical methods for the characterization of enteropeptidase basic biochemical properties electrochemical impedance spectroscopy was used. The solution resistance (R_s) was the main parameter that was studied. One can suppose that after the proteolytic cleavage the conductivity of the solution will change. Therefore, it will be possible to determine the basic biochemical properties of enteropeptidase from the dependence of the conductivity of the solution on the time after the cleavage [1-3].

Basic biochemical characteristics, such as maximum reaction rate (V_{max}), Michaelis constant (K_M) and turnover number (k_{cat}) determined by electrochemical methods, especially by electrochemical impedance spectroscopy will be described. These findings will add to determination of optimal reaction conditions. Comparison between electrochemical results and traditional ones will be also made.

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SDS Influence on the Surface States and Field Effects of n-GaAs(100)

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Surfactants (amphiphilic molecules with a hydrophilic head and a long hydrophobic tail) have been widely applied in electrochemistry to improve the electric and electronic properties of the electrode/solution interface [1-3]. At the electrode surfaces, the surfactants can be arranged in bilayers, cylinders or surface micelles depending on the nature of the electrode surface and the surfactant [4].

GaAs is a promising material for building highly sensitive and fast response devices for chemical and biochemical applications due to its high carrier mobility. However, the presence of large density of surface states makes difficult not only the fabrication of the electronic devices but also affects their functional performances. Since surface states usually originate in the surface impurities and/or surface defects and surfactants are very active agents for removing them, we used such compounds in order to control the surface state densities as well.

The effects of two concentrations of sodium dodecyl sulfate (SDS) (submicellar concentration, 4 mM and micellar concentration, 40mM) on the electronic properties of the n-GaAs(100) electrodes in H_2SO_4 solutions were investigated by electrochemical impedance spectroscopy (EIS). Additional information concerning the influence exerted on their chemical composition and surface morphology was provided by photoelectron X-ray spectroscopy (XPS) and atomic force microscopy (AFM).

The impedance spectra analyzed by using an electrical equivalent circuit considering both the electrical contributions of the semiconducting substrate and the organic overlayer point out that the SDS adsorbed layer changes the population of the electronic surface states localized in the band gap and brings a negative shift of the flat band potential.

XPS results indicate that whereas the Na-1s and S-2p core-levels double their intensity on increasing the SDS concentration, the substrate core-level lines,Ga-3d and As-3d do not exhibit significant changes in their intensity, meaning that the adsorbed layer becomes not thicker but only more compact. Fractal analysis of AFM images shows a smoother and compact surfactant layer for micellar SDS concentration, in agreement with XPS results.

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Microfabricated Electrochemical Sensors for DNA and Protein Assays Using Nanoparticle Labels

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Methods that enable sensitive, selective, and rapid detection of proteins and DNA are important tools in bioanalytical chemistry. The combination of electrochemistry and nanoparticles (metal nanoparticles and quantum dots) serving as electrochemically active labels provides an elegant way to detect DNA and proteins [1-3]. The principle of the detection scheme is to label the target biomolecules with the selected nanoparticles and to convert the labelling nanoparticles to the respective free metal cations which are detected by electrochemical stripping analysis. Gold nanoparticles are the most common labels used in electrochemical detection of proteins and DNA. However, the voltammetric detection of Au(III) (normally performed at carbon-based electrodes) lacks in sensitivity while labelling with gold nanoparticles does not allow multiple detection of more than one biomolecules in a single assay. Quantum dots offer an attractive alternative as electrochemical labels with greater versatility since they allow higher detection sensitivity (by selecting a proper combination of the electrode material and composition of nanocrystals) and the possibility to perform multi-analyte assays in a single run.
On the other hand, microfabrication allows the construction of disposable ready-to-use electrochemical sensors, with reproducible surface, scope for mass production, versatility in the selection of the electrode material and low cost. In this work, some microfabricated sensors are described for the electrochemical assay of biomolecules using nanoparticles labels. Both thin-layer and screen-printed fabrication approaches are explored to demonstrate proof-of-principle detection of DNA and proteins.

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Changes in Protein Structure as Detected by Electrochemical Analysis

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In recent decades, electrochemistry of proteins was limited to relatively small group of conjugated proteins containing non-protein redox centres yielding reversible electrochemistry [1]. We have proposed a new electrochemical method for analysis of practically all proteins, based on the ability of proteins to catalyze hydrogen evolution at mercury electrodes [2,3]. This method, relies on constant current chronopotentiometric stripping and allows protein determination at a much higher sensitivity (down to nanomolar and subnanomolar concentrations) than voltammetric methods. At high stripping current intensities the peak H is sensitive to changes in the protein structures [2], including denaturation [2-4], and aggregation [5], as well as changes resulting from mutations (single amino acid exchange) [6] or changes in the redox state [7].

We applied constant current chronopotentiometric stripping in combination with DTT-modified mercury electrode [8] to study the effect of oncogenic mutations in the DNA-binding domain of the tumor suppressor p53. We observed striking differences between the CPS responses of the wild type protein p53 and its R175H mutant [6]. The CPS responses of wild type and mutant p53 showed excellent correlation with structural and stability data and provided additional insights into the differential dynamic behavior of the proteins.

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Catalytic Hydrogen Evolution of Native and Denatured Proteins at Mercury and Amalgam Electrodes

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In last decades, bare metal electrodes were not widely applied for electrochemical analysis of proteins, since it was believed that adsorption of proteins on metal surfaces led to their irreversible denaturation [1]. Using constant current chronopotentiometric stripping (CPS) peak H it was recently shown that proteins adsorbed at bare mercury electrodes are denatured when exposed to negative potentials but remain native at potentials close to zero charge [2, 3]. Denatured proteins produced well-developed peaks H, which were much larger than those of native forms. Our results showed that the extent of surface denaturation was dependent on experimental conditions, e.g. ionic strength, stripping current (I_{str}) intensity, temperature. The surface-attached proteins did not denature under the usual experimental conditions, i.e. close to neutral pH, moderate ionic strengths and room temperature. At high ionic strengths, the surface denaturation was explained by the effect of strong electric field on the protein immobilized at the negatively charged Hg surface. The time of exposure of the adsorbed protein to negative potentials is related to the I_{str} used in CPS. When negative I_{str} intensities were sufficiently high the time of the exposure to negative potentials was very short and the native structure of adsorbed protein was not affected. On the contrary at low negative I_{str} intensities proteins denatured due to prolonged time exposure to negative potentials. Using peak H it was possible to follow protein unfolding in dependence on experimental conditions, such as ionic strength [4], I_{str} or temperature [5].

Our results suggest that CPS peak H in combination with HMDE can be used in protein structure analysis. Under proper experimental conditions even small structure changes important proteins important in biomedicine can be detected.

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Electrocatalysis in Polyamino Acids and Hexapeptides

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Present fast progress in proteomics opens the door for applications of new methods. Electrochemical methods were shown to be suitable for this purpose. We have shown that almost all proteins and peptides produce peak H due to catalytic hydrogen evolution (CHER) using constant current chronopotentiometric stripping (CPS) at mercury electrodes. This peak was very useful in the analysis of proteins [1,2] such as determination solubility of transmembrane proteins [3], monitoring of protein aggregation [4] and denaturation [1,2], discrimination of protein redox states [5]. For better understanding the peak H nature is helpful to monitor electrochemical responses related to changes in amino acid composition [6-8]. Polyamino acids (polylysine, polyarginine, and polyhistidine) as an intermediate model system between peptides and macromolecular proteins have been investigated to find how different amino acid residues contribute to the catalytic hydrogen evolution reaction at hanging mercury drop electrode. We showed that histidine in polyhistidine behaves [7] as catalysts as well as lysine, arginine in polylysine and polyarginine [6,8]. Disadvantages of polyamino acids are their polydispersed distribution and various structures in analyzed samples. Therefore we chose also monodisperse homo-peptides with well-defined length, as other model system, for better characterization of the contribution of individual amino acid residues to CHER. Here we compared contributions of polyamino acids and their homohexapeptide analogues hexalysine, hexaarginine and hexahistidine to CHER.

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Force and Conductance Measurements in Molecular Switches

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The field of molecular electronics is a branch of nanotechnology that is aimed at fabrication and characterization of electric circuits composed of single-molecule elements. The latter comprise passive (wires, resistors) as well as active (diodes, transistors and switches) electronic components. The presented work focuses on the measurements of single-molecule physical properties of molecular switches. In particular, two particular experimental arrangements are discussed: (1) measurements of nanomechanical properties (interaction forces) in a non-covalent host-guest-based electrochemically driven switch and (2) measurements of electric conductance of an optically addressable switch.

(1) The forces required to detach ferrocene (Fc) guest moiety from β-cyclodextrin (βCD) cavity in individual host-guest complexes were investigated by atomic force microscopy break junction (AFM BJ) technique in an electrochemical environment. The host βCD molecules were self-assembled on a gold-coated AFM probe employing thiol anchoring groups, whereas the ferrocene moieties were immobilized onto the AFM gold(111) substrate by a conductive di(phenylene-ethynylene)thiol linker diluted in an alkanethiol matrix of a variable length. The redox state of the guest Fc moiety as well as the length of the alkanethiol matrix was found to significantly affect the single-complex rupture force, allowing thus the proposed system to function as an electrochemically addressable molecular switch [1].

(2) The conductance properties of a photoswitchable dimethyldihydropyrene (DHP) derivative have been investigated in single-molecule junctions using the mechanically controllable break junction (MCBJ) technique. We demonstrate that the reversible structure changes induced by isomerization of a single bispyridine-substituted DHP molecule are correlated with a large drop of the conductance value. We found a very high ON/OFF ratio ($>10^4$) and an excellent reversibility of conductance switching [2].

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Direct Electrochemical and AFM Detection of Amyloid-β Peptide Aggregation on Basal Plane HOPG

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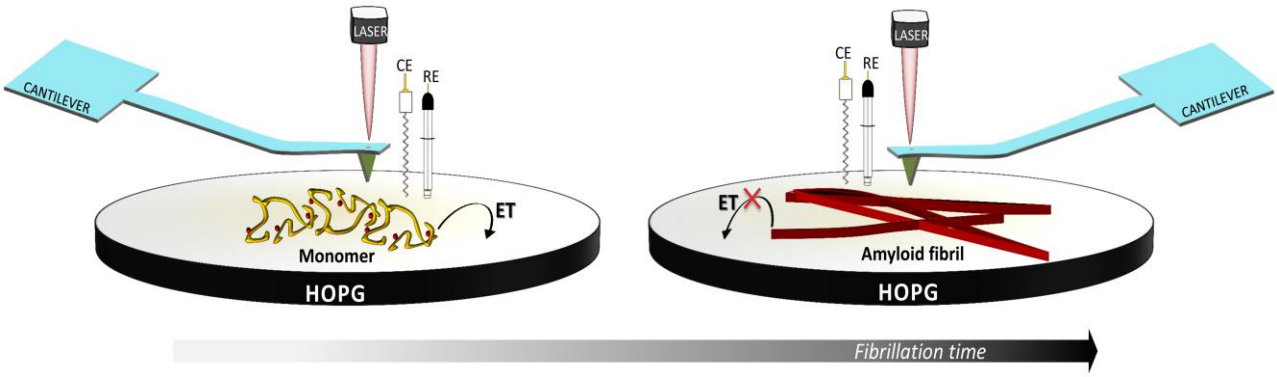
Amyloidogenesis is associated with more than 30 human diseases, including Alzheimer's one related to aggregation of β-amyloid peptide (Aβ) [1]. Aβ aggregation in vitro is commonly studied by such techniques as circular dichroism (CD) spectroscopy, fluorescence spectroscopy, and electron and atomic force microscopy. Electrochemical methods can successfully compete with other techniques in analysis of protein conformational by monitoring electrochemical oxidation of their surface amino-acid residues such as tyrosine [2,3]. Here, consecutive stages of Aβ42 aggregation and amyloid fibril formation were followed electrochemically via oxidation of tyrosines in Aβ42 adsorbed on the basal plane graphite electrode and directly correlated with Aβ42 morphological changes observed by atomic force microscopy at the same substrate. The results offer new tools for analysis of mechanisms of Aβ aggregation.



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Some Aspects of Toxicity of Silver Nanoparticles

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Our ecotoxicology research was focused on ion by modification of the classic eco-toxicological tests used for nanomaterial testing. Regarding previously performed tests of toxicity of nano-silver it is possible to mention for example test on green algae [1], on microorganisms [2] or invertebrate organisms [3]. To prepare a solution of nano-silver, it was possible to use the procedure described [4]. Tests for common chemicals were modified to identify and characterize potential hazards related to nanomaterial behavior. In this case we modified the OECD Test No. 201: Toxicity Test on Embryo and Sac – Fry Stages. Non-stabilized silver nanoparticles with a hydrodynamic diameter of 40 nm were used in the test. The modified eco-toxicological experiment was conducted at four concentration levels (5, 10, 25 and 50 μM) and two sizes of silver nanoparticle agglomerates (200 and 400 nm). With the increase of the total concentration of silver in the solution, a spontaneous gradual growth of nano-silver particles occurred. The analysis showed that the stabilization of their size in the quiet solution was reached after about 80-90 minutes. This stable size was larger, the higher the content of silver in the solution was. The maximum size of the agglomerates was controlled by the periodic exchange of the liquid medium in which the nanomaterial agglomerated. The frequency of fluid replacement was calculated from the agglomeration rate constants, based on data obtained by photon correlation spectroscopy (DLS).
The main idea was experimentally verified on common carp fry. The experiment was evaluated and the contribution of agglomerates with specific sizes on the overall level of ecotoxicity of the studied silver nanoparticles was discussed.

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Acknowledgments

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Gold Nanostructured Surface for Electrochemical Sensing and Biosensing: Does Shape Matter?

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The construction of electrochemical sensor based on gold working electrode modified with gold nanostructures has been achieved via galvanic deposition of gold ions through anodic alumina nanoporous template [1,2]. Two different approaches have been tested to fabricate the nanostructures of various sizes: the first one was a gold deposition using thin alumina template resulting in the production of gold nanorods, and the second one employed thick alumina foil for the production of gold nanowires. The detailed topography of nanostructured surfaces have been analysed by scanning electron microscopy (see Fig. 1). The length and the diameter of nanostructures have a significant influence on active surface area enhancement and thus on the capacity to promote electron-transfer reactions [3,4]. We experimentally studied this dependence by electrochemical impedance spectroscopy and cyclic voltammetry. On the basis of our first results, we speculate that the contribution of shorter gold nanorods to increase of active surface area of electrode is much higher compared to long nanowires.

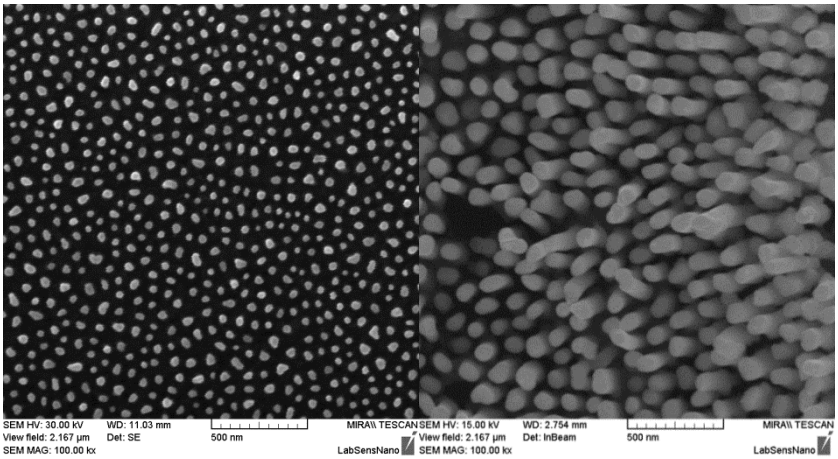

Fig. 1: SEM images of gold nanostructured electrode fabricated via thin alumina template.

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Comparison of Working Electrode Materials for Direct Glucose Oxidation

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Non-enzymatic electrochemical glucose sensors have been researched and developed over the years. These sensors are not only significant for use in blood sugar monitoring, but also in the food industry, bio-processing and in the development of renewable, sustainable fuel cells. A large variety of electrode materials for direct glucose oxidation have been explored including metals (e.g. Au, Pt, Ni, Cu), metal oxides/ semiconductors (e.g., $Ni(OH)_2$, RuO_2, Cu_xO), alloys (e.g. PtRu, PtPb, PtAg, PtAu), complexes (e.g. cobalt phthalocyanine) and carbon based materials (e.g. carbon nanotubes). The process of glucose oxidation using these electrocatalysts is generally described to occur via the adsorption of analyte to the electrode surface. The structure and active surface area of the electrode are therefore also highly important [1-4]. Our previous study has demonstrated that Cu_2O micro/nanoparticles based planar working electrodes can be successfully used to direct glucose oxidation in alkaline medium (see Fig. 1) [5]. Here, we evaluated the behaviour of glucose at planar electrodes based on different materials such as Pt, Au, Cu, Cu_2O and carbon nanotubes.

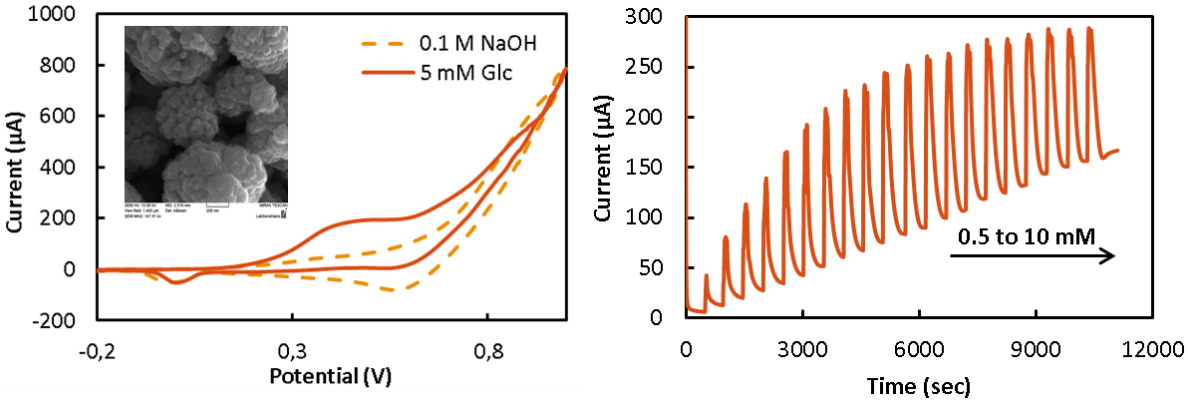

Fig. 1: Cyclic voltammetry response to 5mM glucose on Cu_2O based spray-coated electrode with SEM image of Cu_2O micro/nanoparticles in inset (left) and chronoamperometric response to increasing glucose concentration (right).

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Production and Study of Nanoporous Alumina Membranes by Electrochemical Methods

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Nanoporous alumina membranes manufactured by electrochemical anodization technique are very useful and frequently studied nanostructures. Diameter of obtained nanopores can be controlled by changing conditions during experiments which results in different diameter of nanopores of alumina thin membranes in range of 4 – 250 nm with 10^8-10^{12} pores per cm^2 [1]. Variety of kind nanoporous membranes production allows their use in many branches such as optics, electronics, selective molecule separation, filtration and purification, biosensing, single-molecule detection and template synthesis for manufacturing nanostructured materials [2, 3]. In this study, nanoporous alumina membranes with variables parameters (thickness, nanopores diameter) were made. Then throughput and filtration properties of membranes were characterized by electrochemical impedance and cyclic voltammetry methods.

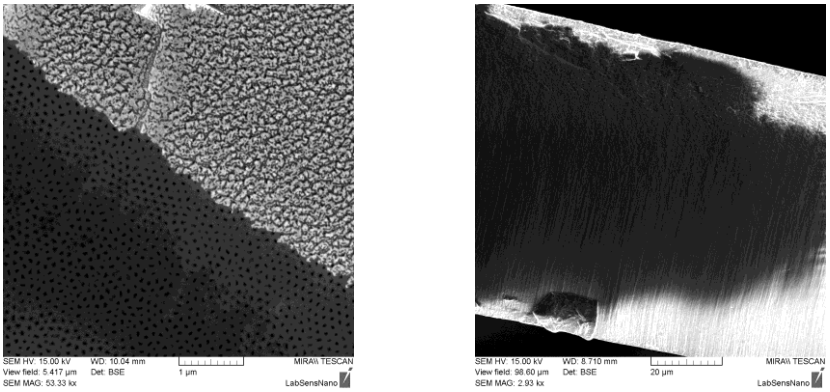

Fig. 1: Nanoporous alumina membranes coated by thin gold film (left top) and pure alumina (left bottom) and cross section (right.)

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Wednesday, May 28th, 2014

Supramolecular Complexation of Biorelevant Analytes by Functional Electroactive Monomers of Thiophene Derivatives for Preparation of Molecularly Imprinted Polymer Films as Recognition Units of Chemical Sensors

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Using a concept of molecular imprinting, we developed a systematic approach to designing and fabricating selective chemical sensors biomimicking recognition of biorelevant analytes [1] including biogenic amines, such as dopamine [2], adrenaline [3], and nicotine [4]. Toward that, we first synthesized several functional and cross-linking monomers of bis(2,2'-bithiophene) derivatives bearing different recognition sites. Then, we computationally modeled structures of self-assembled pre-polymerization complexes of the functional monomers with the analytes, initially used as templates, and calculated thermodynamic parameters of formation of these complexes. By allowing for self-assembly of these complexes in solutions, next, we experimentally confirmed these calculated parameters by determining them with the fluorescent and UV-vis spectroscopy titrations. Subsequently, the conducting analyte templated molecularly imprinted polymers (MIPs) were prepared from these complexes by potentiodynamic electropolymerization, which led to deposition of thin MIP films onto different electrode substrates. After extraction of the templates, monitored by XPS and UV-vis spectroscopy as well as differential pulse voltammetry (DPV) and electrochemical impedance spectroscopy (EIS), vacated molecularly imprinted cavities were left in the films. These cavities, complementary in size and shape to the templating molecules, were capable of selective recognition of the respective analytes. Accessibility of the cavities to the analytes was controlled by suitable adjustment of the film visco-elasticity and porosity with cross-linking monomers and ionic liquids, respectively. With either direct (piezoelectric microgravimetry at a quartz crystal microbalance, capacitive impedimetry) or indirect (DPV) analytical signal transduction, we determined the analytes with the limit of detection at nanomole concentrations. The chemical sensors selectively determined the analytes in the presence of close structural and functional analogues.

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Electrochemical and Spectrophotometric Study of the hydration of Orthophthalaldehyde and Its Reaction with Simple Amines

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Orthophthalaldehyde (OPA) is used for several decades in two main applications: a) as a "pre-column" derivatization agent in fluorescent determination of amino acids (AAs) [1]; b) in hospitals for disinfection of surgery instruments made from plastics [2].
The second above mentioned application is most probably based on the reaction of OPA with primary amine substituents or other nucleophilic centers in nucleic acids, causing cross-linking and change of their tertiary structure and resulting in decomposition of the cell. In literature related to the disinfection procedure we can find many protocols that differ in composition and in recommended time of use. The reason is that this application has been developed empirically because the detailed mechanism of reaction of OPA with amines and other nucleophiles is not completely elucidated. Although the recent studies were focused on the reaction of OPA with ammonia [3], as the simplest amine, due to simultaneous multiple equilibria the identification of intermediates and products was very difficult.
OPA is very slowly soluble in water, but in acetonitrile it dissolves quickly. Therefore the stock solution could be prepared in these two ways. It was found that there is a significant difference in reaction pattern with amines, when using aqueous or non-aqueous stock solution of OPA, respectively. Although the antecedent hydration of OPA was many times reported [4, 5], the dialdehydic form was always considered as the species reacting with amines (generally with nucleophiles). However, the experiments showed that the hydrated OPA reacts with amines directly, whereas the non-hydrated OPA undergoes hydration reaction prior the reaction with amines. This difference is observable also spectrophotometrically. The hydration is pH-dependent: the hydration rate increases with higher pH. The changing course of the reactions of a) OPA with water and b) hydrated/unhydrated OPA with amines under various conditions was evaluated, discussed and the results were mutually compared.
For this investigation DC- and DP-polarography, together with cyclic voltammetry were used. The experiments were performed in aqueous buffered solutions of different pH, in non-aqueous acetonitrile and in mixed acetonitrile/H_2O media. Simultaneously, the reactions were followed by UV/Vis spectrophotometry in order to distinguish heterogeneous and homogeneous principle of investigation and to compare the observed kinetics.

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Electrochemical and Spectrophotometric Study of the Reactivity of Orthophthalaldehyde with Amino Acids

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Reactivity of orthophthalaldehyde (OPA) with nucleophiles is studied for many decades. There are two main applications: analytical determination of amino acids (AAs) based on fluorescent product [1] and disinfection of plastic thermolabile surgery instruments in hospitals [2]. In literature related to the analysis of AAs we can find many protocols that differ in composition and in recommended time of use. The reason is that these applications have been developed empirically because the mechanism of reaction of OPA with nucleophiles is not completely understood.

Recently reaction of OPA with ammonia was studied [3], however, due to multiple equilibria the identification of intermediates and products is very difficult. The present experimental work is focused on a systematic electrochemical and spectrophotometric investigation of the reaction of OPA with simple AAs. After preliminary experiments ten compounds were chosen: eight alkyl based AAs with one primary amino group (glycine, alanine, leucine, isoleucine, valine, norvaline, aminobutyric and aminoisobutyric acid), lysine with two amino groups and glycine ethyl ester preventing zwitterionic equilibrium.

DC-polarography, cyclic voltammetry and a direct recording of i-t curves has proved to be a good tool to observe kinetics of these reactions. Simultaneously, the reactions were followed by UV/Vis spectrophotometry in order to distinguish heterogeneous and homogeneous principle of investigation and to compare the observed kinetics. The measurements were performed in two phosphate buffers with pH above and under the pK_a of amino groups in used AAs and in various proportion of reactants (OPA : AA from 1 : 1 to 1 : 10).

It was found out that relatively slow hydration of OPA occurs prior the investigated reaction with the amino group. The kinetics of the reactions was evaluated and discussed. The products were identified by MS (fig. 1). The main results of this study are: 1) the reactive species of OPA is not its unhydrated dialdehydic form, as expected in the literature [4, 5], but its hydrated form; 2) the AAs with quaternary α-carbon (aminoisobutyric acid) show negligible reactivity with OPA; and 3) the reaction rate is higher at higher pH.

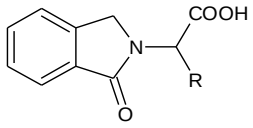

Fig. 1: Product of reaction of OPA with a simple amino acid.

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Electroanalysis of Antioxidants in Surfactant Micellar Media

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Antioxidants are one of the important types of analytes in life sciences. Being easily oxidizable compounds, they are often investigated using electrochemical methods. Surfactants are widely used in analytical chemistry including voltammetry in the organized systems that provides better analytical characteristics of determination as well as solubilization of lipophilic analytes in water media. From other side, surfactants modify the electrode surface changing its properties and consequently reaction rates and pathways. Thus, the electroanalysis of antioxidants in surfactant micellar media is of interest.

Voltammetric behavior of phenolic antioxidant eugenol and redox mediator menadione (Fig. 1) has been investigated on glassy carbon electrode (GCE) in surfactant micellar media.

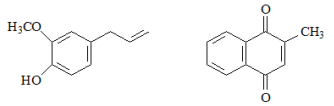

Fig. 1: Eugenol and menadione structure.

Eugenol is irreversibly oxidized on GCE at 780 and 700 mV in 0.1 M $LiClO_4$ in 0.1 M Triton X100 and Brij® 35 micellar media, respectively. Electrochemical oxidation of eugenol is irreversible diffusion-controlled process and involves 2.0±0.1 electrons corresponding to formation of *o*-quinone. The eugenol calibration graph is linear in the range of 15-1230 μM with the estimated detection limit of 3.8 μM and the quantification limit – 12.6 μM. The addition of ethanol (10% v/v) to 0.1 M Triton X100 micellar media leads to cathodic shift of eugenol oxidation potential on 50 mV. Under these conditions, the oxidation current linearly depends on eugenol concentration in the range of 0.02-1.0 mM with the detection limit of 0.01 mM. The recovery of eugenol determination in test solutions is in the range of 99.0-101.2%. The preliminary extraction of eugenol with ethanol is used for its voltammetric determination in spices. Quantitative determination of eugenol in essential oils in Triton X100 micellar media has been carried out.

Menadione cyclic voltammograms show pair of redox steps on GCE in 0.1 M H_3PO_4 with potential separation of 343 mV. Cationic, nonionic and anionic surfactants micellar media significantly decrease the menadione peak potential separation. Statistically significant increase of menadione reduction current (3- and 4.4-fold) has been observed in Triton X100 and sodium dodecyl sulfate (SDS) micellar media, respectively. Electrochemical reduction of menadione in 9 mM SDS micellar media is reversible diffusion-controlled one-electron process corresponding to formation of relatively stable semiquinone anion radical. The linear dynamic ranges of menadione determination are 7-560 and 600-2550 μM with the limits of detection and quantification of 1.66 and 5.53 μM, respectively. The voltammetric method for the determination of menadione in pharmaceutical "Aekol" based on preliminary extraction with ethanol has been developed.

Thus, surfactant micellar media provide solubilization of lipophilic analytes in water media and significantly change the forms and characteristics of cyclic voltammograms leading to the improvement of analytical characteristics of antioxidants.

Acknowledgments

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Electron Transfer Initiated Bond Cleavage. Beyond the *ECE*

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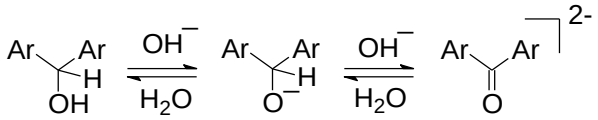
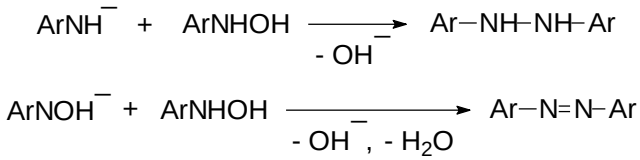
The electrochemical reductive cleavage of the bonds in organic compounds starts as *ECE* process and results in formation of two anions, what enable one to anticipate the reaction of proton transfer and/or nucleophilic substitution between the species and initial compound. A complex of experimental (cyclic voltammetry, chronoamperometry, electrolysis) and theoretical methods (digital simulation, quantum chemical calculations) was employed to study the processes using as an example OH-acids electroreduction in aprotic solvents.

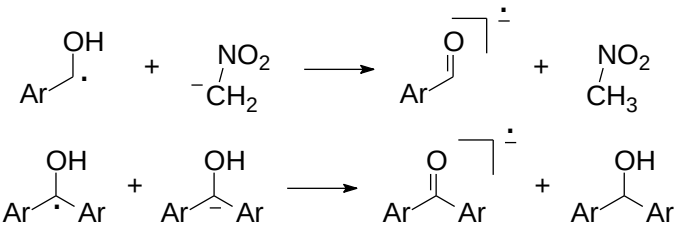
The results obtained show that cathodically generated anion radical of aryl methanol derivatives can undergoes both hydroxide anion elimination and C-C bond cleavage. It was found that hydroxide anion abstracts protons both from the initial compound and from its anion giving rise corresponding π*-dianion:



Unlike aryl methanol case, electroreduction of phenyl hydroxylamine derivatives produces only monoanions of aniline and hydroxylamine derivatives. Both of them are involved in the competing nucleophilic substitution reactions affording hydrazine- and azo-compounds:



The C-C bond cleavage takes place when it results in π-anion formation. This reaction we observed for 1-phenyl-2-nitroethanol, fluorenpinacol and benzopinacol. In contrast with the previous case of C-O bond cleavage, the anion is protonated not by starting alcohol but by its radical:



The proton transfer between these species affords anion radical of carbonyl compound. Electron transfer from the last to the parent alcohol initiates the cyclic process.

The thermodynamics and kinetics of all processes mentioned above have been investigated by quantum chemical and electroanalytical methods.

Acknowledgments

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Electrochemical Study of New Triazaborine Based Compounds

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This contribution represents a fundamental electrochemical investigation of a series of recently synthesized compounds [1,2] based on triazaborine core (see Fig.1). The main attention has been paid to redox characterization of these molecules, to the determination of the first oxidation and reduction potential and to the localization of reaction centers. For this study [3] in non-aqueous *N*,*N*-dimethylformamide polarography, cyclic voltammetry and rotating disk voltammetry were used. In the homologous series the first reduction proceeds as a one-electron reversible process localized at the -N=C-C=N- part of the central heterocycle being in conjugation with the attached carbonyl. The first oxidation of triazaborines proceeds as a two-electron irreversible process, most probably of the ECE type, localized at the negatively charged boron atom and surrounding unsaturated structures including the substituted phenyl ring. For better understanding of the relationship between the structure and redox properties, the approach using sigma (para) constants of Hammett type was used. The energies of the longest-wavelength absorption bands taken from UV-vis spectra were compared with the experimentally found differences E_{ox}–E_{red} and with calculated HOMO-LUMO gaps. The calculated optimized structures and localization of the frontier orbitals confirmed the interpretations. The results will be used for tuning of properties and for design of the next generation of triazaborine compounds.

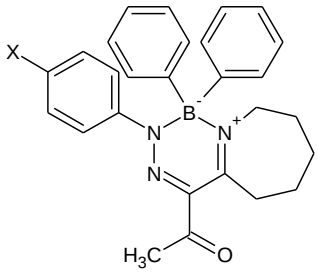

Fig. 1: Structure of triazaborine derivatives

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Acknowledgments

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Oxidation of Bioactive Flavonoid Taxifolin in Nonaqueous Media

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The flavonoid taxifolin is included in the family of flavanones [1]. Taxifolin exhibits anti-inflammatory effect [2] by acting as the antioxidant and was used as a drug against leukocyte activation [3].

This study deals with the oxidation mechanism of taxifolin in non-aqueous solutions, which is not yet elucidated. The electrochemical oxidation of taxifolin in 0.1 $mol \cdot L^{-1}$ $TBAPF_6$ in acetonitrile or dimethyl sulfoxide was performed on glassy carbon electrode using cyclic voltammetry (CV). CV showed two oxidation waves up to the potential 2.1 V. The first oxidation wave was irreversible and the electrode reaction was controlled by diffusion. The charge consumption during bulk electrolysis corresponded to the transfer of two electrons at the potential of the first oxidation wave.

In order to identify oxidative products of taxifolin, the UV-Vis and IR spectroelectrochemistry, HPLC-DAD and HPLC-MS were performed. IR-spectroelectrochemistry confirmed oxidation of hydroxyl groups in the B-ring and o-quinone formation at the potential of the first oxidation wave. Several oxidation and degradation products of taxifolin were confirmed by HPLC-MS/MS and HPLC-DAD. Benzofuranon was one of the main products found after the electrolysis of taxifolin in DMSO.

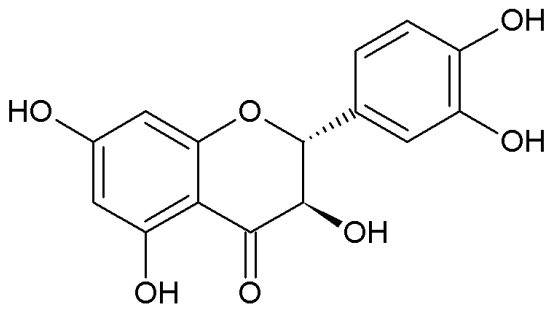

Fig. 1: Taxifolin structure

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Structure-Redox Reactivity Relationship in a Series of Extended Pyridinium Compounds

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The electron-withdrawing and electron-donating properties of extended and expanded pyridinium molecules are extensively studied because of their potential applications in the fields of molecular electronics [1]. The characteristics of expanded branched pyridinium cations depend strongly on their chemical structure. We have shown recently that two-electron reduction of selected expanded branched pyridinium cations can proceed either in a single step or stepwise depending on the steric constraint around the *N*-pyridinio site [2].This work is focused on the elucidation of the reduction mechanism of four expanded branched pyridinium cations: 1',3',5'-trimethyl-2,4,6-triphenyl-1,4'-bipyridine-1,1'-diium (**1**), 1'-methyl-2,4,6-triphenyl-1,4'-bipyridine-1,1'-diium (**2**), 1',3,5-trimethyl-2,4,6-triphenyl-1,4'-bipyridine-1,1'-diium (**3**) and 1'-methyl-2,3,4,5,6-pentaphenyl-1,4'-bipyridine-1,1'-diium (**4**). The properties of the compounds were studied by DC, AC polarography and electrochemical impedance spectroscopy (EIS) in dimethyl sulfoxide in order to suppress adsorption.

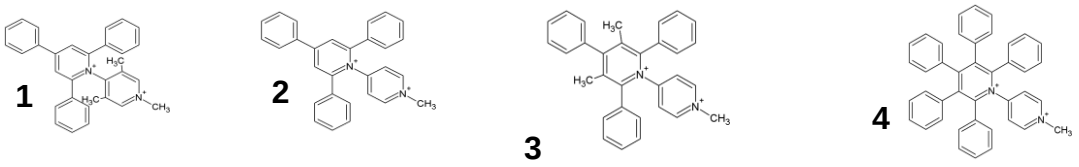

Fig. 1: Studied molecules

Compound **1** is reduced in two separate one-electron steps, whereas compounds **2** to **4** undergo a single two-electron reduction. Interestingly, the heterogeneous rate constant of the first electron transfer of molecule **1** (measured by EIS) is higher than the values obtained for molecules **2** to **4**. Molecule **1** is a representant of the class of molecules that allow for only minimal structural change on the pyridinium moiety upon the electron transfer process. In this work we discuss the possible correlation between the observation of the potential compression/inversion, electron transfer rate parameters and the ability of the molecule to undergo structural changes upon reduction.

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How electroanalytical Techniques Can Be Used in Complexation Studies of Heavy Metals with Biomolecules

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Heavy metals can cause serious damages, even at very low doses, by replacing essential elements on biological functions. Plants, algae, fungi, and mammalians have developed natural mechanisms to be protected of the action of these metals. The mechanisms are based in the intracellular synthesis of Cys-rich polypeptides that complex heavy metals making them innocuous for the organism. These peptides, in the case of plants, algae and fungi are phytochelatins (PC_n) that have the general structure $(\gamma\text{-Glu-Cys})_n\text{-Gly}$, where *n* usually ranges between 2 and 5. In the case of mammalians, heavy metal regulation is through metallothioneins (MT) that are also proteins with high content in thiol groups (around 30% of cysteine content). Although the capability of these compounds for complexing metal ions is well known, the sequence of formation and the final stoichiometries of the different complexes involved have needed especial attention.

Voltammetric techniques have always been considered very convenient tools to study complexation processes. However, in many cases the postulation of a theoretical physicochemical model is very difficult because the electrode process, the transport phenomena process or both of them are rather involved. An alternative global approach can come from Chemometrics [1,2]. This approach is based on extracting results and/or identifying models from numerical and statistical analysis of the data, instead of fitting an assumed *a priori* theoretical model to the experimental data. Thus, the combined use of voltammetric techniques and chemometric methods as multivariate curve resolution with alternating least squares (MCR-ALS) or gaussian peak adjustment (GPA) has proved to be very useful for the study of heavy metal complexation by a variety of naturally occurring ligands.

The usual method is based on voltammetric titrations of a metal solution with the considered ligand and *vice versa*, using differential pulse voltammetry (DPV) that provides signals for the different species of the system: free polypeptide, free metal ion and metal bound in different chemical environments. The subsequent analysis of the experimental DPV data matrices allows the characterization of involved systems taking profit of the great capability of this approach. These measurements can be done not only in mercury electrodes but also in bismuth electrodes or in modified screen printed electrodes [3].

The competition between either heavy metal ions or ligands and the determination of complexing capacities can be studied, providing valuable information to interpret phenomena occurring in natural samples. Concerning the determination of these complexes in natural samples, a simple, sensitive and cheap method that uses HPLC with amperometric detection has been proposed.

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Behavior of Metallothioneins, their Fragment and Phytochelatin at Mercury and Amalgam Electrodes

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Using hanging mercury drop electrode, numerous studies were performed with mammalian metallothioneins (MT) containing mainly bound cadmium and zinc [1]. Originally, two metal binding domains are recognized, where 60-61 amino acids (containing 20 cysteinyl groups) form two-clusters structure, with seven divalent metal ions tetracoordinated by sulfur: α- Me_4 S_{11} a β-Me_3 S_9. In solution, the changes connected with pH or excessive metal ions occur and can be studied with voltammetric methods on HMDE, which can distinguish Cd (II) or Zn(II) coordinated by one, two or four sulfur atoms, having its reduction peak at different potentials. Methods of elimination voltammetry showed adsorption of tertracoordinated complexes on mercury electrode, whereas adsorptive stripping chronopotentiometry followed changes of inert complexes to complexes with labile behavior [2, 3]. As a model for such structural changes were cadmium or zinc complexes with phytochelatin PC_2, where multivariate curve resolution with alternating least squares (MCR –ALS) has been applied [4].
Nevertheless, there are many fields, where only total metallothionein concentration is searched. In such cases, there is a choice between immunochemical methods and electrochemical Brdička reaction, where hanging mercury electrode is employed [5]. Brdička reaction with Co (III) in ammonia solution is very sensitive with the application of constant current chronopotentiometry and especially with adsorptive transfer method [6]. Silver solid amalgam electrodes under condition of Brdička reaction were successfully tested with phytochelatin [7] but were not successful with MT. The reason is the formation of Ag-MT complex, which we showed on HMDE, using the addition of silver ions to the metallothionein solution. Similarly, formation of Ag-PC complex after addition of silver ions to the solution of phytochelatin PC_2 was detected. Conditions were found for determination of rabbit liver metallothionein Cd_5Zn_2MT, fragment Lys-Cys-Thr_Cys-Cys-Ala and phytochelatin PC_2 at CuSAE [8] and AgSAE amalgam electrodes. In extracts from biological material, the interference of zinc in comparable concentration should be avoided.

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Transport of Biochemically Important Ions and Compounds across Biomimetic Membranes

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A large number of biologically important ions and compounds are coming into contact with plants, animals or men. Such compounds can be transported into these organisms, more precisely, into their cells, and after entering cells, into their subcellular structures (e.g., from cytosol to vacuole). Simultaneously, they are transported into different parts of organism (e.g., from plant roots to leaves). More precisely, any species taking part in metabolic processes must be first transported across the biological membranes.
The latest results in elucidation transport processes of charged species across the biomimetic membranes will be presented in this contribution. The attention has been paid to the transport of hazardous metals (e.g., Cu, Cd) in free form as well as in form of their complexes (e.g., with low molecular weight organic acids, phytochelatins). Three different ways of preparation of biomimetic membranes, composed of simple phospholipids (PLs) (e.g., lecithin) have been realized: liposomes, self-assembling PL membranes (PLMs) on the agar surface [1], and PLMs in pores of polycarbonate substrate [2-5]. The biomimetic membranes composed of lecithin and cholesterol have been studied too [6].
Firstly, the real transporters were replaced by fat-soluble polypeptides [5]. Later, the transport processes have been studied using real membranes (protoplasts isolated from leaves of tobacco, potato, and barley). Finally, the real membranes mixed with model PLMs have been investigated. The PLMs and the transporting processes were characterized by electrochemical methods (electrochemical impedance spectroscopy (EIS), voltammetry, ion selective electrodes) as well as by non-electrochemical methods (optical microscopy, AFM, electrospray ionization mass spectrometry (ESI-MS) [5]).

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Optimization the Cell Wall Degrading Enzymes and Technique for Isolation of Protoplasts in Potato

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Plant organisms have ability to communicate with the surrounding environment and maintain homeostasis to properly function. This is the reason why it is necessary to create the optimal conditions for plant growth and then understand the mechanism of compounds transport across membranes. In our experiments, the plant materials must be definited and characterized to optimize procedure for isolation of protoplasts. Protoplasts are useful tools to study the transport of macromolecules and production of somatic hybrids. Plasma membrane of plant cells is surrounded by cellulose wall and adjacent cells are joined together by a thick pectin rich matrix. Separation of plant cells and removal of the cell wall experimentally, by either a mechanical or an enzymatic process, results in the production of protoplasts [1]. Protoplasts can be obtained from all types of actively growing young and healthy tissues. The most convenient and widely used source of plant protoplasts is the leaf. Juvenile seedling tissues, cotyledons are other alternative tissues most frequently used for protoplasts isolation [2]. All the environmental and genotypic factors, which affect the cell wall thickenings and compactness indirectly, influence the number of protoplasts recovered. Protoplasts are isolated by two methods, mechanical and enzymatic. The enzyme mixture solution of cellulose/macerozyme is used to digest the cell wall. The critical factors affecting the obtaning of protoplasts are the kinds of cell wall degrading enzymes, the physiological state of plant leaves, the type of osmotic stabilizers and the composition of reaction solution. With the improvement of technique and enzyme combination rate, the yield of collected protoplasts will be increased higher.

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Preparation of Plant Material for the Study of Membranes by Electrochemical Methods

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Plant cell in comparison to the animal cell is characterized by the formation of cell wall. The cell wall removing from plant cell by enzymes is important for the research of plant membranes. The sterile condition must be used during all the time of preparation. These conditions will ensure that the plant cell is not contaminated with undesired organisms such as fungi and bacteria. In our experiments we used the potato cultivar Bintje, cultivated at *in vitro* conditions on Shenk and Hildebrandt Medium. The mesophyll of leaf was used for obtaining the protoplasts [1] Potato leaves were cut up into small strips and then were stored in the solution of the enzyme mixture of 1g cellulase Onozuka R10 and 0.25g R10 macerosyme dissolved in 100 ml W5 solution. Release of protoplasts was carried out in the dark at 25 degrees of Celsius after 18 hours. Liquid phase was filtered through a sieve of 70 to 90 microns, and centrifuged for 5 minutes at 800 rpm. The supernatant was discarded and the pellet was resuspended in W5 solution. Centrifugation was performed for 5 minutes at 800 rpm for the second time. The supernatant was discarded and the pellet was resuspended in 4 ml of 20 percent sucrose solution and overlayed by 2 ml of W5. The next centrifugation was performed for 10 minutes at 400 rpm. The floating protoplasts were transferred into the W5 solution. Throughout, it was necessary to work carefully with protoplasts, without impacts and sudden movements in order to prevent damage of protoplasts. Then the protoplast was prepared for electrochemical studies.

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Biomimetic Electroanalytical Potentiometric Sensing System Utilizing Interface of Two Immiscible Electrolytes

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We develop fast potentiometric sensor utilizing interface of two immiscible electrolyte solutions (ITIES) which imitates the potential response of semipermeable biological membrane. This type of sensing system can be used as stationary sensor, but also in flow analysis.
In the presented work the water/1,2–dichlorobenzene interface was formed, with aqueous solvent as mobile phase and organic liquid as the stationary phase. The sensing properties are based on phase transfer redox reaction with phthalocyanine acting as a mediator in nonaqueous phase and with tetrabutylammonium hexafluorophosphate as phase transfer agent. The employed phthalocyanines undergo the redox reaction with analyte and contribute to regeneration of the detection system. This type of potentiometric sensor based on ITIES appears to be highly resistant to saturation compared to solid electrodes.
Reductive analytes such as SH-containing compounds (e.g. sulphides, cysteine etc) can be detected in open-to-air conditions due to competitive regeneration reaction of phthalocyanine-mediator with oxygen, establishing the equilibrium interface potential. Characteristic E-t response of different model analytes reflects the specific interaction taking place on the interface, which can be utilized for their selective detection.

Acknowledgments
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Electrochemical Study of Fisetin

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The natural flavonoid compound fisetin (2-(3,4-dihydroxyphenyl)-3,7-dihydroxychromen-4-one) is a bioactive flavonoid compound present in plants, seeds, fruit, vegetables, such as strawberries, blueberries, apples, grapes, cucumbers and onions [1,2].
Compound is important for its antioxidative, anti-carcinogenic and anti-inflammatory properties [3,4]. The dying properties have an impact in plants pigmentation and were already used successfully for coloring of tapestries in the 15th and the 16th century.

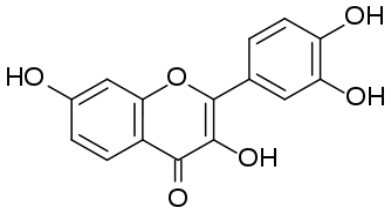

Fig. 1: Chemical structure of fisetin.

This study is focused on the determination of oxidation pathways of fisetin and identification of its degradation products. It is based on cyclic voltammetry and UV/Vis spectroelectrochemistry in aqueous media. The distribution of the degradation products during the electrolysis in aqueous media was monitored by HPLC-MS and HPLC-DAD analysis. Fisetin is unstable when exposed to atmospheric oxygen, which causes degradation and complicates their analytical determinations, so the amount of oxidative reaction products increases with the time of exposure to the air [5,6].
Our results underline the importance of the electrochemical methods in the clarification of the oxidation processes of bioactive molecules.

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Acknowledgment

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On the Oxidation of Drug Diflunisal in Non-aqueous Media

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Diflunisal (DIF) is a synthetic difluorophenyl derivative of salicylic acid and presents similar analgesic and anti-inflammatory activity. It belongs to the non-steroidal anti-inflammatory drug class (NSAID). It is used to treat moderate pain and relieve the inflammation, swelling and joint pain associated with rheumatoid arthritis and ostearthis [1]. Recently, it is used to treat amyloid diseases.

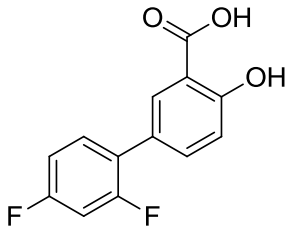

Fig. 1: Chemical structure of diflunisal.

Several methods have been reported for the assay of diflunisal in its formulations and biological fluids [2,3]. To our knowledge, no previously studies have been done about its mechanisms of oxidation.

The electrochemical oxidation of diflunisal in 0.1 mol·L^{-1} $TBAPF_6$ in acetonitrile was studied on a glassy carbon electrode. Diflunisal yields one irreversible oxidation wave at 1.6 V (*vs.* Ag/AgCl/1 M LiCl electrode) and the electrode reaction is controlled by diffusion. The influence of the basicity of the solvent was studied by measurements of cyclic voltammograms at different concentration of pyridine. The oxidation mechanism depends on the presence of dissociation forms in solution. The oxidation peak at 1.6 V decreases and a new peak at 1.11 V increases with the increasing concentration of pyridine. There is no significant shift of their potentials, which indicates that carboxylic group is not involved in the oxidation. The overall oxidation mechanism of dianion of diflunisal involves the participation of hydrogen.

The study is based on cyclic voltammetry, electroanalytical methods and UV-Vis spectroelectrochemistry. The oxidation products were determined by separation techniques (HPLC-DAD, GC-MS).

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Green Electrochemical Sensors Based on Boron Doped Diamond and Silver Amalgam for Sensitive Voltammetric Determination of Antineoplastic Agent Methotrexate

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Methotrexate (MTX) is an antimetabolite and antifolate drug used in treatment of a cancer and autoimmune diseases. It acts by inhibiting the metabolism of folic acid. Methotrexate was originally used as a part of combination chemotherapy regimens to treat many kinds of cancers. It is still the mainstay for the treatment of many neoplastic disorders including acute lymphoblastic leukaemia. The structure of MTX is analogous to folic acid, it differs only in a methyl group connected to the amino group of amino benzoic acid (N(10)) and the amino group which is substituted on C4 on pyridine circle (Fig. 1).
The voltammetric behavior of methotrexate using unmodified boron-doped diamond electrode (BDDE) [1], mercury meniscus modified (m-AgSAE) and polished silver solid amalgam electrode (p-AgSAE) [2] is described in the present paper. Optimum working conditions for differential pulse voltammetric determination of MTX were found and proposed sensitive methods were employed in the analyses of pharmaceutical preparations.

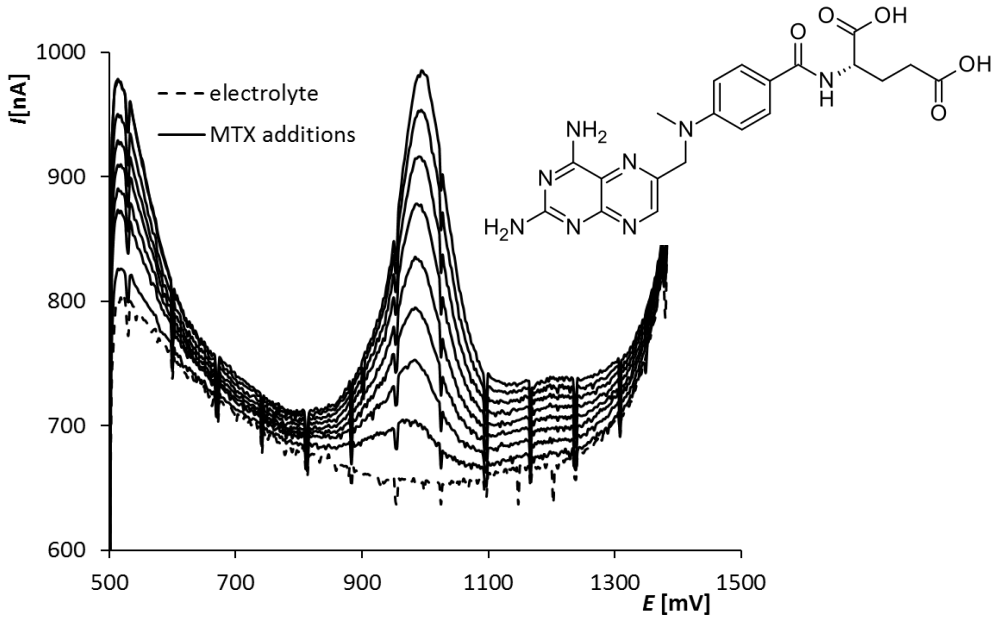

Fig. 1: Concentration dependence of methotrexate recorded on BDDE

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Acknowledgments

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Voltammetric Study of Ion and Electron Transfer from Water to Highly Hydrophobic Ionic Liquids: Electroanalytical Aspects

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Cyclic voltammetric (CV) studies of ion transfer and electron transfer across the interface between an ionic liquid (IL) and an aqueous electrolyte solution (w) have indicated the possible applications of this approach in ion electroanalysis [1]. Such application relies on the resolution of voltammetric responses of various ions in the test aqueous solution. The selectivity of the polarized IL-w interfaces is determined by the standard ion transfer potential or the standard Gibbs energy of ion transfer, which can be modified by a suitable ligand forming stable complex with the target ion [2]. In this contribution we shall compare the ion selectivity for several ILs composed of tridodecylmethylammonium ($TDMA^+$) or redox-active (ferrocenylmethyl)dodecyl- dimethyl ammonium ($FcMDDA^+$) cations and tetrakis[3,5-bis(trifluoromethyl)phenyl]borate ($TFPB^-$), tetrakis (pentafluorophenyl)borate ($TPFPB^-$) or redox-active Co dicarbollide ($CoDCC^-$) anions. Comparison is based on the CV measurements of the ion transfer using the electrochemical cell with a single-interface polarized membrane (SIPM), e.g.,

$$Ag`|AgCl|\ RCl\ (w')\ |R^+\ S^-\ (m)|\ LiCl,\ I^{+/-}\ (w)\ |AgCl|Ag \qquad (1)$$

or with a two-interface polarized membrane (TIPM), e.g.,

$$Ag`|AgCl\ |LiCl(w')|R^+\ S^-(m)\ |LiCl,\ I^{+/-}(w)|\ AgCl|Ag \qquad (2)$$

where $I^{+/-}$ is the target ion, the aqueous phases are denoted by w and w', and m denotes the membrane phase with the IL cation R^+ and the IL anion S^-. IL membrane is supported on a thin (ca. 110 μm) micro-porous filter [3]. An analysis of the CV data makes it possible to establish a linear Gibbs energy relationship for ion transfer from water to IL and to an organic solvent immiscible with water such as 1,2-dichlorobenzene [3,4]. CVs also have used to examine the transfer of alkali metal cations, proton and ammonium ion facilitated by the complex formation with valinomycin at the polarized IL-w interface [5]. Apart from the evaluation of the complex stability constants in IL, this study has demonstrated a good resolution of voltammetric responses of K^+ and Na^+ in the presence of an excess of Mg^{2+} or Ca^{2+}. Similarly, respecting possible analytical applications, have been studied transfers of electrons [6], transfers of polyions [7] and of anions [8].

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Determination of 5-Nitroindazole using Silver Solid Amalgam Electrode

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Nitro-group containing compounds have been frequently studied by electrochemical techniques. These are mostly based on the reduction of the nitro group at the aromatic or heterocyclic ring [1, 2].

The voltammetric behavior of 5-nitroindazole (5-NI) was investigated by differential pulse voltammetry (DPV) and cyclic voltammetry (CV). The mercury meniscus modified (m-AgSAE) and polished (p-AgSAE) silver solid amalgam electrodes (both of inner diameter 0.5 mm) [3, 4] were used as the working electrodes. Britton-Robinson buffer was used as the supporting electrolyte. The reaction mechanism was investigated using CV and elimination voltammetry with linear scan (EVLS). DPV with optimized working parameters was utilized for analysis of model solutions containing 5-NI. The limits of detection were calculated as 0.14 μmol L for m-AgSAE and 0.47 μmol L for p-AgSAE, respectively. The optimized proposed method was successfully applied in analysis of real water spiked by 5-NI.

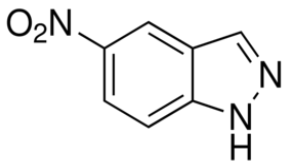

Fig. 1: Structure of 5-nitroindazole

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Acknowledgments

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Author index

Metrohm
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Iontová analýza pod jednou střechou

- **Iontová chromatografie**
- **Titrace**
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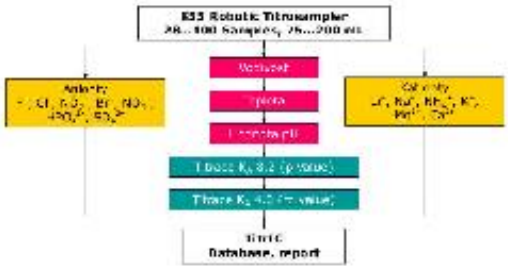
Metrohm Česká republika s.r.o. je dceřinou společností švýcarské firmy Metrohm AG. Metrohm má více jak 70 let zkušeností s iontovými analýzami. Od prvních jednoduchých pH metrů až k současným plně automatizovaným a sofistikovaným systémům, pro maximální zjednodušení Vaší práce v laboratoři.

Jako jediný nabízíme spojení různých technik iontové analýzy do výkonných analytických řešení, jako např. spojení iontové chromatografie a titrace (**TitrIC**) nebo iontové chromatografie a voltametrické analýzy (**VoltIC**).

Ukázka systému TitrIC III pro:



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