XVII. WORKSHOP OF PHYSICAL CHEMISTS AND ELECTROCHEMISTS

BRNO, 30TH AND 31ST MAY, 2017

BOOK OF ABSTRACTS

XVII. Workshop of Physical Chemists and Electrochemists

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611 37 Brno

http://www.sci.muni.cz

THE ORGANIZATIONAL SECURITY OF THE CONFERENCE

Libuše Trnková

libuse@chemi.muni.cz

(Department of Chemistry, Faculty of Science, Masaryk University)

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An introductory word...

We would like to kindly invite you to the conference **XVII. Workshop of Physical Chemists and Electrochemists** which is held at Masaryk University in two days, 30th and 31st May. The conference is devoted to new aspects in physical, biophysical and electroanalytical chemistry. Due to recent events we have decided to include in our program the lectures that will be dedicated to Dr. Michael Heyrovský and Doc. Pavel Kubáček. Both contributed to the development of chemical discipline as an exact and perspective part of scientific research, Michael Heyrovský in the field of bioelectrochemistry and bioanalysis, Pavel Kubáček in the field of organic physical chemistry, including the questions of structures of organic molecules and their reactivity. During the conference two posters describing their professional life and their successes will be highlighted. According to this program you can see the schedule of lectures and other events. The conference days are opened with the series of plenary lectures, followed by the invited lectures. The afternoon time in both days is devoted to competitive lectures in the "Young Scientists' Session". All participants are invited to the Poster Session followed by the gala dinner on Tuesday afternoon. On Wednesday, 31st of May, the conference will be concluded with the announcement of three winners of the "Young Scientists' Session" and one winner of the Poster Session. We believe that you will welcome the opportunity to present and discuss your scientific and educational results.

Enjoy the conference!

Libuše Trnková

Motto:

„What is the creative process in science? The ability to know what's important and what's secondary."

Jaroslav Heyrovský

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DNA APTAMERS: STRUCTURE, PHYSICAL PROPERTIES AND BIOSENSING APPLICATIONS

Tibor HIANIK[1*], Alexandra POTURNAYOVÁ[1,2], Lenka BÁBELOVÁ[2], Monika BURÍKOVÁ[3], Jozef BIZÍK[3], Michael LEITNER[4], Constanze LAMPRECHT[5], Andreas EBNER[5]

[1] *Department of Nuclear Physics and Biophysics, Faculty of Mathematics, Physics and Informatics, Comenius University, Mlynská dolina F1, 842 48 Bratislava, Slovakia*

[2] *Center of Biosciences of the Slovak Academy of Sciences, Institute of Animal Biochemistry and Genetics, Dúbravská cesta 9, 840 05 Bratislava, Slovakia*

[3] *Biomedical Center of the Slovak Academy of Sciences, Institute of Experimental Oncology, Dúbravská cesta 9, 845 05 Bratislava, Slovakia*

[4] *Center for Advanced Bioanalysis GmbH, Gruberstrasse 40, 4020 Linz, Austria*

[5] *Institute of Biophysics, Johannes Kepler University Linz, Gruberstrasse 40, 4020 Linz, Austria*

**tibor.hianik@fmph.uniba.sk*

Aptamers are single stranded DNA or RNA molecules that are selected *in vitro* by SELEX (Systematic Evolution of Ligands by EXponencial enrichment) [1] toward various targets, such as proteins, drugs, biomarkers, viruses, bacteria or whole cells. In a solution aptamers fold into 3D structure containing binding site for the target. The affinity of aptamers to their targets is in the micromolar to the subnanomolar range which is comparable and in certain cases even better than, those of antibodies for the same targets. Aptamers are considered as artificial antibodies. However, in comparison with antibodies they are more stable and flexible. Once the aptamer sequence is developed this can be reproduced with high accuracy. Aptamers can be chemically modified by various labels, that allowing them to be immobilized at various surfaces at which serve as receptors in biosensors [2]. The chemical modification also increases the aptamer stability. Electroactive and optical probes can be conjugated to aptamers, which allowing signal amplification using various methods [3]. AFM tips modified by aptamers allowing measurement of the forces between aptamer and its target at the solid support or at the surface of the cell [4]. This contribution introduces into the structure and physical properties of DNA aptamers and shows applications of aptamers in development of biosensors and in the study of aptamer ligand interactions.

ACKNOWLEDGEMENT

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DOES GANGLIOSIDE GM1 PROMOTE NEURODEGENERATION OR DOES IT ACT NEUROPROTECTIVE? MOLECULAR INSIGHTS FROM SINGLE MOLECULE FLUORESCENCE

Martin HOF

J. Heyrovský Institute of Physical Chemistry, Czech Academy of Sciences, Prague, Czech Republic.

http://hof-fluorescence-group.weebly.com/

Oligomers of the β-amyloid (Aβ) peptide are thought to be implicated in Alzheimer's disease (AD). Membrane in neurons may mediate the oligomerization of Aβ present in brain. Using the single-molecule sensitivity of fluorescence, we address the oligomerization of Aβ monomers on lipid bilayers containing essential components of the neuronal plasma membrane. We find that Sphingomyelin triggers the oligomerization of Aβ and that physiological levels of GM1, organized in nanodomains, do not seed oligomerization. Moreover, GM1 prevents oligomerization of Aβ counteracting the effect of Sphingomyelin. Our results establish a preventive role of GM1 in the oligomerization of Aβ suggesting that decreasing levels of GM1 in brain, e.g. due to aging, could lead to reduced protection from the oligomerization of Aβ and contribute to AD's onset [1].

In addition to the new insights into the molecular mechanism(s) that may be involved in AD, it should be pointed out that this work contains a further important novel finding. We uncovered the existence of nanoscopic heterogeneities (radius 8-26 nm) in microscopically homogenous membranes, unresolvable by super-resolution microscopy. This was achieved by a combination of Monte Carlo Simulations, FLIM-FRET [2] and FCS techniques [3] using our recently developed fluorescent ganglioside analogues [4].

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CHARGE TRANSPORT IN SINGLE MOLECULE JUNCTIONS

Magdaléna HROMADOVÁ[1*], Štěpánka LACHMANOVÁ[1], Viliam KOLIVOŠKA[1], Jakub ŠEBERA[1], Jindřich GASIOR[1], Gábor MÉSZÁROS[2], Philippe P. LAINÉ[3], Michal VALÁŠEK[4], Marcel MAYOR[4]

1 *Department of Molecular Electrochemistry, J. Heyrovský Institute of Physical Chemistry of CAS, v.v.i., Dolejškova 3, 182 23 Prague 8, Czech Republic*

2 *Research Center for Natural Sciences, HAS, Magyar tudósok krt. 2, H-1117 Budapest, Hungary*

3 *University Paris Diderot, 15 rue J.-A. de Baïf, UMR CNRS 7086, 75013 Paris, France*

4 *Karlsruhe Institute of Technology (KIT), Institute of Nanotechnology, P.O. Box 3640, 76021 Karlsruhe, Germany*

**hromadom@jh-inst.cas.cz*

Molecular electronics suggests the use of molecules as an alternative to silicon–based technology. Molecules are more versatile and allow further miniaturization of electronic devices. Performance of such devices can be tuned more efficiently by changes in the chemical structure of molecules. Commercially available molecular electronic devices are becoming a reality.[1,2]

This contribution deals with charge transport properties of single molecule junctions of several model systems[3–8] obtained by scanning tunneling microscopy break junction technique (see Figure below). One group of molecules does not contain redox active centers and was synthetized for efficient anchoring of single molecules of desired functionality to the conducting substrate. They contain molecular wire and a tripodal anchor. Several aspects of charge transport mechanism like conduction pathway, type and number of anchoring groups will be discussed. Break junction measurements will be complemented by quantum mechanical calculations of the junction evolution geometries and single molecule conductance values.

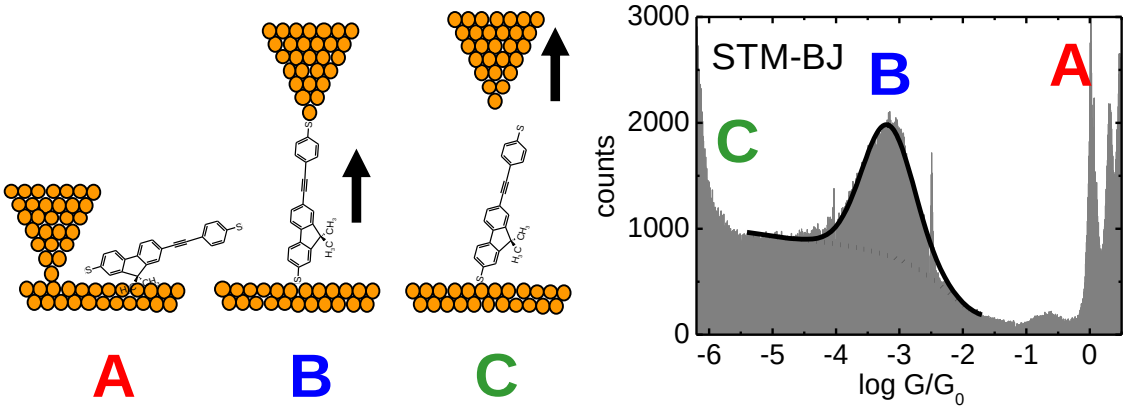

Figure: Principle of the Scanning Tunneling Microscopy Break Junction Technique

Second group of molecules contains pyridinium-based redox centers. Molecules with multiple pyridinium units were designed to probe the relationship between length and single molecule conductance and their representatives can function as high-conductance molecular wires. Molecules containing one pyridinium redox center are suitable for comparison of their charge transfer properties in the electrochemical sense as described by Marcus theory and charge transport properties in metal-molecule-metal junctions within the framework of Landauer scattering approach. Relationship between electron transfer rates and zero-bias molecular conductance of non-adiabatic redox systems was treated theoretically by Nitzan and Ratner.[9]

Experimental findings for pyridinium-based redox systems are being discussed in view of this recent theoretical treatment.

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ANTICANCER METALLODRUGS: MECHANISTIC INSIGHTS

Jana KAŠPÁRKOVÁ[1,2 *], Viktor BRABEC[1,2]

[1] *Institute of Biophysics, Academy of Sciences of the Czech Republic, v.v.i., Kralovopolska 135, 61265 Brno, Czech Republic*

[2] *Department of Biophysics, Faculty of Science, Palacky University, 17. listopadu 12, 77146 Olomouc, Czech Republic*

**jana@ibp.cz*

Despite the widespread use of antineoplastic platinum drugs in the clinic, a number of accompanying disadvantages exist. In connection with attempts to circumvent these problems new metallodrugs have been prepared and mechanisms underlying their biological activity have been extensively investigated. These mechanistic investigations involve research focused on understanding interactions of metallodrugs with DNA since DNA binding and recognition of DNA modified by the metallodrugs are the important processes responsible for their anticancer properties. I will discuss how various classes of metallodrugs, which can interact with DNA can alter the cellular response induced by conventional platinum drugs used in the clinic. I will describe that these alterations can be achieved by changing: (i) the nature and structure of the DNA lesion induced; (ii) conformational alterations induced in DNA by these lesions; and (iii) various cellular signaling pathways initiated by metallodrug-DNA damage. We anticipate that summarization of the results on DNA binding of cytotoxic metallodrugs may help to shed light on their potency and will make it possible to create new strategies to design rationally new anticancer metal-based compounds.

ACKNOWLEDGEMENT

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ELECTROCHEMISTRY OF PROTEIN-LIPID MEMBRANE COMPLEXES

Jan VACEK*

Department of Medical Chemistry and Biochemistry, Faculty of Medicine and Dentistry, Palacky University, Hnevotinska 3, 775 15 Olomouc, Czech Republic,

**jan.vacek@upol.cz*

Membrane proteins (MPs) are proteins associated with the membranes of a cell or separated organelles. It has been estimated that ~30% of all genes in most genomes encode MPs. For this reason, MPs are interesting target molecules in current biochemical research and hence the search for novel methodologies for studying MPs is important in the investigation of their structure and function. In this contribution, a procedure for measuring the intrinsic electroactivity of MPs using mercury and carbon electrodes is described. MPs were analysed after their (a) solubilisation by detergents or selected ionic liquids [1-6] and/or (b) incorporation into liposomes or more complex lipidic systems (*e.g.* lipidic cubic phases) [7-9]. The formed species, solubilized proteins (*cf.* a) or complex lipid-protein asociates (*cf.* b), were adsorbed onto electrode surfaces and typical anodic and cathodic protein peaks were monitored. The electrochemical responses reflected the concentration of the studied MPs in the samples and the changes in electrochemical peaks indicate the structural changes in the MPs resulting from ligands binding to them. Na/K ATPase (ubiquitous, sodium/potassium pump), uncoupling proteins (components of mitochondrial membranes), protein ftt1103 (bacterial periplasmic protein) and cytochrome *c* were used as model proteins. The applicability of intrinsic electroactivity measurement for research on lipid-protein interactions is discussed in general, especially in the context of studying the interactions of MPs with detergents, ionic liquids, solid surfaces and binding ligands such as drugs and toxins

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ELECTROCHEMICAL AND ELECTROPHORETIC DEPOSITION OF MATERIALS OBTAINED BY ANODIC DISSOLUTION OF METALS IN WATER

Jan HRBÁČ[1,2], Jan ROZSYPAL[2], Daniel RIMAN[2], Vladimir HALOUZKA[2], Dušan HEMZAL[3] and Vít PAVELKA[1]

1 *Department of Chemistry, Faculty of Science, Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic*

2 *Department of Analytical Chemistry, Palacky University, Faculty of Science, 17. listopadu 12, 771 46 Olomouc, Czech Republic.*

3 *Department of Condensed Matter Physics, Faculty of Science, Masaryk University,*

* *jhrbac@atlas.cz*

Electrochemical dissolution of metal anodes in unsupported medium such as water generates metal oxide/hydroxide material which can be deposited onto a suitable substrate connected as a cathode in the electrochemical cell. Depending on the properties of anode-derived material (zeta potential and solubility), metal electroreduction or electrophoretic deposition of anode-derived material can be achieved. Nanostructured deposits can be applied as amperometric and SERS sensors. In the previous works[1-3], we showed that anodization of silver and copper in ultrapure water leads to Ag^+ resp. Cu^{2+} cations, reacting with OH^- ions available from the autoprotolysis of water to give corresponding hydroxides, eventually transformed by further hydrolytic reactions. This anode-derived material is transported in the interelectrode space by electrophoretic, diffusion and convection induced by density or temperature gradients. After reaching the surface of the counterelectrode (cathode), a deposit is formed. Recently, the above described strategy was used also to deposit nickel and copper-copper nickel alloy onto carbon fiber microelectrodes. In the case of silver and copper the procedure leads to elemental silver resp. copper while material obtained from nickel and copper-nickel alloy is of oxidic nature, as suggested by EDX. We therefore assume, that silver and copper deposits are created indirectly by Volmer-Weber electrochemical reduction of silver(I) resp. copper(I)/(II) free cations which exist in equillibrium with insoluble anode-derived oxidic material. On the other hand, it appears that after initial period of Volmer-Weber growth, the deposition of nickel and copper-nickel alloys proceeds electrophoretically leading to thick and homogeneous layer of nickel resp. copper/nickel oxide. We hypothesize that the deposition mode depends on zeta potential of the anode-derived material which might be negative in the case of silver and copper or positive for nickel and copper-nickel. This quantity is, however, experimentally not accessible in unsupported medium. The layers prepared using the above described deposition procedures are applicable in the area of electrochemical and optical (SERS) sensing, depending on the nature of deposited material.

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COMPUTATIONAL THERMODYNAMICS - TOOL FOR DEVELOPMENT OF ADVANCED MATERIALS

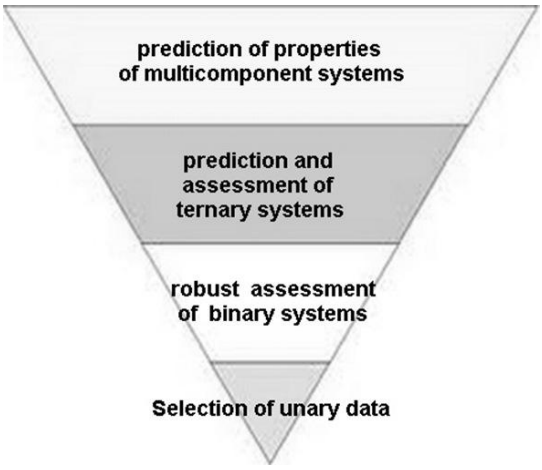
Aleš KROUPA[1]

1 *Institute of Physics of Materials, CAS, Žižkova 22, 616 62 Brno, Czech Republic*

INTRODUCTION

The understanding of phase diagrams and phase equilibria is crucial for the development of new materials and understanding of their materials properties. Basically, the material properties of alloys are influenced by the microstructure, which is governed by the material composition and heat treatment. The final microstructure can be deduced from the knowledge of relevant phase diagrams and treatment, both during material preparation and its exploitation. Therefore phase diagram is essential tool for prediction which phases should be expected for given conditions (temperature, pressure, overall composition of the alloy). Even if the material in question is not in the equilibrium state, the metastable phase diagram can be established in some cases and the knowledge of equilibrium phase diagram can offer the insight into the processes, which can be expected during the exploitation of the material.

The phase diagrams are based on the knowledge of thermodynamic properties of the materials, and as soon as they are established for simpler systems, there is a possibility to calculate phase diagrams even for complex systems. Nevertheless, such possibility requires powerful computers and development of suitable theoretical methods; therefore, until recently, the experimental studies of phase diagrams prevailed. The results of the phase diagram experimental studies have been published in various phase diagram compendia both in the form of collected phase diagrams, e.g. [1] or in the form of critically assessed evaluations of existing results (e.g. [2,3]). The experimental approach is still necessary, especially for simpler systems, but it is time consuming and expensive. The complexity of modern materials makes practical application of experimental studies very difficult. Therefore small understanding of predictive possibilities of phase diagrams existed among researchers working in the field of applied research. First attempts to apply a theoretical approach to the problem of the phase diagrams and thermodynamic properties modelling can be traced deep into 20th century, nevertheless first practical method was developed in seventies. The background of the semi-empirical *CALPHAD* method was laid by Kaufman as well as Hillert and their co-workers [5–7] and the practical use of the theoretical modelling started with the development of several software packages e.g. [8,9]. The basic principle of the *CALPHAD* method is shown in the Figure. It is based on very robust theoretical assessment of binary systems and ternary based on existing experimental data. Using physically sound models for the description of Gibbs energies of all phases existing in the systems, it allows prediction of thermodynamic properties of very complex systems corresponding to advanced materials currently being developed. The software packages together with the thermodynamic databases now allow successful modelling of the phase equilibria and relevant sections of phase diagrams for such materials and important material properties can be deducted from such information.



The modelling of phase diagrams and thermodynamic properties offers not only valuable information in the field of the equilibrium thermodynamics, but it can be used as useful source of data for multiscale modelling, as modelling of diffusion processes in multicomponent systems [8], prediction of physical, mechanical and chemical properties of alloy, modelling of grain growth and processes on the interface, and using phase field method [10].

The *CALPHAD* method allows users to decrease significantly the extent of experimental work. It is possible to test theoretically large number of compositions in the search for materials with favourable properties. The CALPHAD method is currently the most widely used for the applications in basic and applied research and in material development in industry. The basic principles of the CALPHAD methods will be described in the lecture, together with examples of its application, strengths and weaknesses, its prediction capabilities etc. Also the basic information about existing software packages, thermodynamic databases and rules for their development will be mentioned here.

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ULTRASENSITIVE H_2O_2 SENSING BY $TI_3C_2T_X$ MXENE MODIFIED GLASSY CARBON ELECTRODE

Lenka LORENCOVA[1], Tomas BERTOK[1], Erika DOSEKOVA[1], Alena HOLAZOVA[1], Jaroslav FILIP[2], Peter KASAK[3], Jan TKAC[1*]

1 *Institute of Chemistry, Slovak Academy of Sciences, Dubravska cesta 9, Bratislava 845 38, Slovak Republic*

2 *Department of Environment Protection Engineering, Tomas Bata University in Zlin, Vavreckova 275, Zlin 762 72, Czech Republic*

3 *Center for Advanced Materials, Qatar University, P.O. Box 2713, Doha, Qatar*

**Jan.Tkac@savba.sk*

It is shown, that the $Ti_3C_2T_x$ can be applied for electrochemical reactions in cathodic potential window for ultrasensitive detection of H_2O_2 down to nM level with a response time of ~10 s.

MXenes belong to a family of exfoliated transition metal carbides and carbonitrides synthesized by hydrofluoric acid (HF) etching of the "A" group element from "MAX" phase powders [1] (where "M" is a transition metal, "A" is an element mostly from groups 13 and 14 of a periodic table, and "X" is a carbon or a nitrogen atom [2] resulting in 2D layered structure similar to graphenes [3]. MXenes are commonly applied as an electrode material for batteries [4], supercapacitors [5] and for electromagnetic shielding [6].

In this study we aimed at electrochemical performance of $Ti_3C_2T_x$ in an aqueous solution for sensing applications. The $Ti_3C_2T_x$ was characterized by means of contact angle measurement, Raman Spectroscopy (RS), Atomic Force Microscopy (AFM), X-ray Diffraction (XRD), X-ray Photoelectron Microscopy (XPS) and Secondary Ion Mass Spectrometry (SIMS). SEM images revealed formation of micrometer size aggregates (Figure).

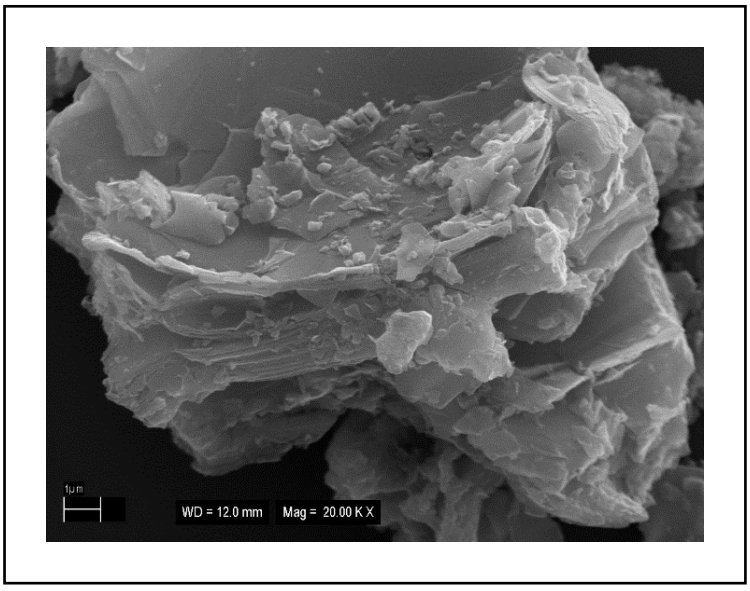

Figure: Representative SEM image of $Ti_3C_2T_x$ sonicated for 1 min. Magnification: 20,000×.

Reduction of H_2O_2 on $Ti_3C_2T_x$ modified electrode started with an onset potential of 160 mV, comparable to the results obtained previously on either chemically reduced GO [7] or carbon nanotube modified electrode [8]. Thus, it can be concluded that $Ti_3C_2T_x$ modified electrodes could be applied in oxidase-based biosensing as effectively as graphene-based devices.

The calculated limit of detection (LOD) for the sensor prior to H_2O_2 addition was as low as 0.7 nM and after taking into account noise level of 150 nA, after addition of H_2O_2, the calculated LOD was 3.5 nM. The sensor exhibited sensitivity of detection of 596 mA cm^{-2}

mM^{-1} with a detection response time of 10 s. The H_2O_2 sensor based on $Ti_3C_2T_x$ showed much higher sensitivity compared to previously published H_2O_2 sensors with sensitivity up to 1.08 mA mM^{-1} cm^{-2} and a LOD of 20 nM [9].

The study showed that exposure of $Ti_3C_2T_x$ to an anodic potential induces formation of TiO_2, which is subsequently most likely etched from the $Ti_3C_2T_x$ surface by present F^- ions. However, pristine $Ti_3C_2T_x$ could be effectively applied in a cathodic potential window for sensing purposes. Results suggested that $Ti_3C_2T_x$ exhibits low catalytic activity for oxygen reduction reaction (ORR) run either in acidic or alkaline media, but $Ti_3C_2T_x$ was proved as an excellent catalyst for reduction of H_2O_2

The H_2O_2 sensor based on $Ti_3C_2T_x$ is the most sensitive device described so far with a detection limit of 0.7 nM comparable to the best device described so far (i.e. 0.3 nM) [10]. It is possible that further modification of $Ti_3C_2T_x$ by metallic nanoparticles could further enhance performance of modified $Ti_3C_2T_x$ to detect H_2O_2.

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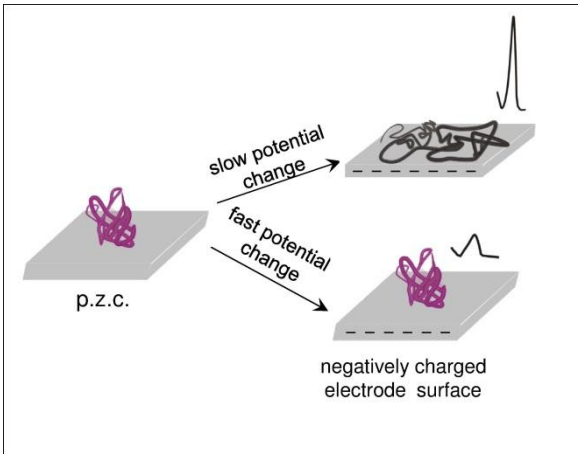
INTERACTION OF PROTEINS WITH SURFACES VIEWED BY CHRONOPOTENTIOMETRIC STRIPPING ANALYSIS

Veronika OSTATNÁ*, Hana ČERNOCKÁ, Veronika KASALOVÁ, Emil PALEČEK
Institute of Biophysics of the CAS, v. v. i., Královopolská 135, 612 65 Brno, Czech Republic
*ostatna@ibp.cz

In memory of Dr. Michael Heyrovský

Proteins were the first biomacromolecules analysed by electrochemical methods. In 1930, only 8 years after J. Heyrovský's invention of polarography, Heyrovský and Babička published their results showing that albumins, in presence of ammonium ions, produced the direct current polarographic "presodium wave", for which catalytic evolution of hydrogen was responsible [1]. We studied peptides and proteins using constant current chronopotentiometric stripping (CPS) [2] peak H (in tribute to J. **H**eyrovsky, **H**ydrogen evolution and **H**igh sensitivity) instead of polarographic presodium wave [3, 4] at bare and thiol-modified mercury-containing electrodes almost 15 years. The electrocatalytic evolution of hydrogen at mercury electrodes depends on contact/adsorption of the catalyst with the electrode surface. Proteins dynamically interact with the electrode surface in dependence on current density, which influences time of exposure of the catalysing molecule to electric field effects [5].



Scheme 1 Effect of I_{str} intensities (current densities) on CPS response of native serum albumin. In CPS the rate of potential changes increases non-linearly with current density. Serum albumin does not show any sign of denaturation close to the potential of zero charge (p.z.c.), however denatured after its exposure to negative potentials.

CPS peak H is well separated from the background discharge and displays sensitivity to local and global changes in protein structure at mercury electrodes [6]. This peak was applied in the analysis of (i) free proteins, such as tumor suppressor p53 [7], anterior gradient protein AGR2 [8] involved in cancer, α-synuclein and peptides involved in neurodegenerative diseases [6], membrane proteins as well as of (ii) proteins in their complexes with DNA [9] and proteins [10]. Obtained chronopotentiometric results were in good agreement with results generated by other methods such as fluorescence, dynamic light scattering, gel electrophoresis, H/D exchange mass spectroscopy etc. Our results show new possibilities in electrochemical analysis of practically any protein.

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FROM POLAROGRAPHY TO ELIMINATION VOLTAMMETRY

Libuše TRNKOVÁ

Department of Chemistry, Faculty of Science, Masaryk University, Kotlarska 2, 611 37 Brno, Czech Republic
**libuse@chemi.muni.cz, litrn@seznam.cz*

In memory of Dr. Michael Heyrovsky († 12. 04. 2017)

Thirty years ago the theory of elimination polarography (EP) and elimination voltammetry with linear scan (EVLS) was firstly published and experimentally verified [1-6]. The elimination procedure applied in both polarography as well as voltammetry can be considered as a mathematical model of the transformation of current-potential curves capable of eliminating some selected current components, while conserving others by means of elimination functions. While the elimination functions in EP use the differential dependence of a current component on time, the EVLS works on the basis of the different dependence of current components (diffusion, charging and kinetic) on the scan rate. Thereafter, the chosen EVLS function needs two or three voltammetric (LSV or CV) curves measured at different scan rates only. Due to longer time and experimental demands of elimination polarography, EVLS has been achieving greater development and usage during last decade. To this date it has found applications not only in electroanalysis, but also in studying electrode processes of inorganic and organic electroactive substances at mercury, silver and/or graphite electrodes [7-35].

For fully adsorbed electroactive species the function eliminating charging and kinetic current components, and conserving the diffusion current component, yields the specific, sensitive and well developed peak-counterpeak (p-cp) signal [7,8,16]. This signal, usually 10-20 times higher than corresponding measured voltammetric peak, is successfully employed in the analysis of nucleic acids and short homo- or hetero-deoxyoligonucleotides (ODNs) containing adenine and cytosine [10,15,17,19,21,22,33,34]. Moreover, it has been shown that the EVLS in combination with adsorptive stripping procedure is a promising tool for achieving very good resolution of electrode processes, for qualitative and quantitative analysis of ODNs and their components, as well as for the identification of ODN structures [10,15,17,19,21,22,33,34]. EVLS increases the current sensitivity for nucleic bases resolution and, thus, for the recognition of base sequences in ODN chains. Using EVLS the adsorptive state of ODNs was also proved in chemoreversible oxidation of guanine moieties after their previous reduction proceeding at negative potentials [33,36].

The lecture will summarize successful applications of EVLS in the electroanalysis of biomolecules, especially of nucleic acids and their components. The pros and cons will be discussed with special attention to capabilities of EVLS: (a) to detect the processes hidden in the predominant current, such as the discharge of the supporting electrolyte, (b) to increase significantly the sensitivity of voltammetry, (c) to determine the charge transfer coefficient, and (d) to detect a chemical reaction preceding the electron transfer. Thus, the EVLS offers a new tool contributing to better understanding of basic electrochemical processes on an electrode surface and being capable to markedly improve the sensitivity of voltammetric assays.

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MOLECULARLY IMPRINTED POLYMERS – (BIO)ANALYTICAL PLATFORM WITH WIDE RANGE OF APPLIACTIONS

Tereza VANECKOVA[1], Marketa VACULOVICOVA[1,2] and Vojtěch ADAM[1,2*]

[1] *Department of Chemistry and Biochemistry, Mendel University in Brno, Zemedelska 1, 613 00 Brno, Czech Republic*

2 *Central European Institute of Technology, Brno University of Technology, Purkynova 123, 612 00 Brno, Czech Republic*

**marketa.vaculovicova@mendelu.cz*

ABSTRACT

Technology of molecularly imprinted polymers (MIPs) is enabling to overcome the obstacles involved in utilization of antibodies and aptamers. These involve mainly the high price because of the difficulties connected with their acquirement. Moreover, the crossreactivity may be an issue. Especially due to the variability, versatility, and diversity, number of MIP applications is steadily grooving over last decades.

The lecture will cover the main principle, highlight the pros and cons, and summarize the most recent applications in bioanalysis.

INTRODUCTION

Development of chemical and biochemical sensors involves an interdisciplinary research combining the knowledge of chemists, biochemists, molecular biologists, and engineers. Generally, biomolecules including antibodies, aptamers, and enzymes are used as recognition moieties. Such molecules have a very high affinity for target analytes; however, they may be un-stable, the affinity diminishes significantly under *in vitro* conditions, and they involve high costs. On the other hand, molecular imprinting is a method involving artificial recognition sites that memorize the shape and chemical properties of the analyte in synthetic polymer. Molecularly imprinted polymers (MIPs) are beneficial over the natural molecules due to their greater chemical stability, lower costs, and simplicity of preparation.

Although creating a MIP against small molecules or peptides are straightforward now, imprinting of large structures, such as proteins and other biomacromolecules (viruses, bacteriea) are still somewhat challenging. From the application point of view, MIPs are widely used in sample pretreatment and chromatographic separation (solid phase extraction, monolithic column chromatography, etc.) and sensing (electrochemical sensing, fluorescence sensing, etc.) of active molecules, pharmaceuticals, environmental pollutants and so on.

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EXPERIMENTAL STUDY OF Al-Cu-Zn PHASE DIAGRAM

Ondřej ZOBAČ[1*], Aleš KROUPA[1], Klaus RICHTER[2]

[1] *Institute of Physics of Materials, The Czech Academy of Sciences, Žižkova 22, 616 62 Brno, Czech Republic*

[2] *Department of Inorganic Chemistry - functional materials, University of Vienna, Althanstraße 14, 1090 Vienna, Austria*

zobac@ipm.cz

Al-Cu-Zn systems have been heavily investigated due to their importance to industry (e.g. automotive engine components, aircraft). The β phase with BCC_A2 structure exhibits the shape-memory properties. The basic thermodynamic descriptions, based on the CALPHAD approach, of the relevant binary phase diagrams were presented in the COST 507 [1] project. All of the binary systems have been investigated intensively in the past and extensive experimental results and theoretically calculated data are available [5,6]. Ghosh et al. [2] published a review of the experimental studies on the Al–Cu–Zn ternary phase diagram published up to 2002. In all of the assessed isothermal sections from 350 °C to 700 °C in [2], the γ_phase region is continuous from the Al–Cu side (γ_Cu9Al4, *cP52*) to the Cu–Zn side (γ_Cu5Zn8, *cI52*). Liang and Schmid-Fetzer [3] did not accept the complete solid solution range of the γ_phase in spite of older theoretical prediction of Al-Cu-Zn phase diagrams [6]. They described this decision in detail in the text of their paper. The design of our experimental description of this system was focused on questions which have not been satisfactorily resolved yet in the scientific literature and on the divergent results from different authors [3, 6]. Figure 1 shows comparison of three phase diagrams published by different authors.

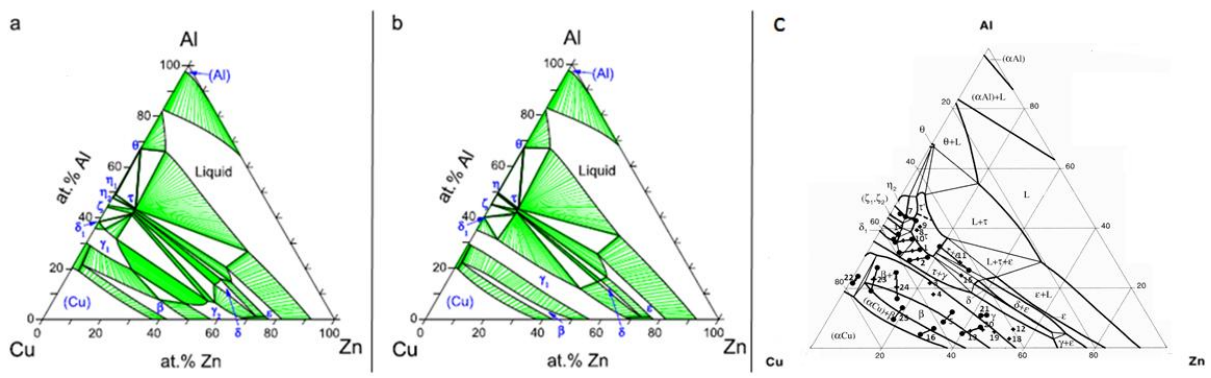

Figure 1: Isothermal section of Al-Cu-Zn phase diagram at 550 °C a) theoretical phase diagram with two-phase field [6] b) theoretical phase diagram with the γ_phase region is continuous from the Al–Cu side to the Cu–Zn side [3] c) published experimental phase diagram [2] with superimposed our experimental results

The overall composition of the samples was chosen with regard to the published sections of the Al–Cu–Zn phase diagram. The Al-Cu-Zn alloys were prepared from pure metals in quartz ampoules. Samples were remelted at 900 °C several times and homogenized by manual shaking. Long term annealing was performed in evacuated quartz glass ampoules employing a sintered alumina crucible to avoid direct contact of metallic aluminum with the quartz glass ampoule to omit contamination of the samples by silicon oxide.

Long-term annealed samples at given temperature were experimentally investigated by combination of various static and dynamic analytical methods. Overall and phase composition was studied using SEM-EDX and TEM electron microscopy techniques. Structure determination and the phase composition was determined from X-ray powder diffraction pattern. More accurate structure determination was done by single crystal XRD methods. Temperature of phase transitions was analyzed by DTA methods in evacuated quartz ampoules under the inert atmosphere of argon. Heating and cooling rate was 5 K min^{-1}. Our work was focused on the description of the region of mutual coexistence of γ_AlCu and γ_CuZn phases and ternary intermetallic phases τ and τ'.

Different results in comparison with published data [2] were found for studied temperature. We described two-phase field γ_AlCu and γ_CuZn but it is much thinner than Liang predicted. Shape of some phase fields are different in comparison with experimental phase diagram published by Ghosh [2].

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DETERMINATION OF THE DIMER-MONOMER EQUILIBRIUM OF THE 14-3-3 PROTEIN

Tomáš BROM[1], Zuzana TROŠANOVÁ[1], Petr LOUŠA[1], Veronika WEISOVÁ[1], Gabriel ŽOLDÁK[2], Jozef HRITZ[1*]

1 *Central European Institute of Technology, Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic*

2 *Technical University Munich, James-Franck-Straße 1, 85748 Garching bei München, Germany*

* hritz@ceitec.muni.cz

14-3-3 proteins are family of regulatory proteins affecting various biological processes in eukaryotic organisms. We can find seven isoforms of 14-3-3 proteins in human cells and up to 15 in plants. They play significant role in regulation of cell growth, apoptosis, cytoskeletal dynamics and transcriptional control of genes expression though influencing the key signaling proteins. Functions of 14-3-3 proteins are mainly dependent on dimeric structure. 14-3-3 proteins can form homodimers as well as heterodimers in equilibrium with monomers. Equilibrium can be shifted to monomer under certain stress conditions. Studies showed that monomers keep binding activity towards phosphorylated partners, they act as heat-shock proteins and they possess chaperone-like activity. Determination of dimer-monomer equilibrium is essential to fully understand the functions of 14-3-3 proteins.

In our study, we prepared a new construct of 14-3-3ζ protein with a single solvent accessible cysteine at the N terminal end. This cysteine was used for attachment of one fluorescent dye, containing maleimide group, to one 14-3-3 monomeric unit. Firstly, protein was labelled separately by AlexaFluor 488 (donor) and AlexaFluor 647 (acceptor). Mixing of acceptor and donor labeled 14-3-3 protein leads to time dependent increase of Förster resonance Energy Transfer (FRET) signal. This phenomenon was used for determination of thermodynamic parameter (Kd) as well as kinetic parameters (kon/off).

Dependence of these parameters on temperature, salt concentration and pH was measured.

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SYNTHESIS OF COMPOSITES OF GRAPHENE OXIDE WITH METAL NANOPARTICLES AND THEIR ANTIMICROBIAL ACTIVITY

Zuzana BYTEŠNÍKOVÁ[1], Lukáš RICHTERA[1,2], David HYNEK[1,2], Pavel KOPEL[1,2] and Vojtěch ADAM[1,2*]

[1] *Department of Chemistry and Biochemistry, Faculty of Agronomy, Mendel University in Brno, Zemedelska 1, 613 00 Brno, Czech Republic*

2 *Central European Institute of Technology, Brno University of Technology, Purkynova 123, 612 00 Brno, Czech Republic*

**vojtech.adam@mendelu.cz*

ABSTRACT

This paper deals with synthesis of antimicrobial composites of graphene oxide with metal nanoparticles. Graphene oxide (GO) has been prepared by modified Hummers' method and characterized using SEM, IR spectroscopy and elementary analysis. There has been synthesized composite of GO with metal nanoparticles which has been characterized. Potential antimicrobial activity of the nanocomposites was tested against *Escherichia coli*, *Staphylococcus aureus* and MRSA.

INTRODUCTION

GO is a modified form of graphene, which contains exogenous oxygen-containing functional groups on its surface. The functional groups allow the application of wide spectrum of substrates to form thin layers or networks [1,2]. Researchers are searching for new antibacterial agents due to the outbreak of infectious diseases. New antimicrobial agents and materials have to be synthesized for the treatment of resistant bacterial diseases. There is a real perceived need for the discovery of new compounds showing antimicrobial activities. The newly synthesized compounds should be more effective and could act through a specific mechanism of well-known classes of antimicrobial agents which many clinical pathogens are now resistant [3]. GO as well as metal nanoparticles exhibit wide antimicrobial activity against pathogenic bacteria [4-6]. Graphene with antimicrobial effect is used for the covering of biomedical equipment or in food packaging, where the bacterial colonization of a surface is undesirable [7].

MATERIAL AND METHODS

Preparation of graphene oxide

The GO was prepared by chemical oxidation of 5.0 g graphite flakes (Sigma-Aldrich, and 100 mesh, ≥75% min) in a mixture of concentrated H_2SO_4 (670 mL, Sigma-Aldrich, ACS reagent 95.0%–98.0%) and 30.0 g $KMnO_4$ (Sigma-Aldrich > 99%) according to the simplified Hummer's method [8]. The reaction mixture was stirred vigorously. After 4 days, the oxidation of graphite was terminated by addition of H_2O_2 solution (250 mL, 30 wt% in H_2O, Sigma-Aldrich, ACS reagent). Formed graphite oxide was washed 3 times with 1 M HCl (37 wt% in H_2O, Sigma-Aldrich, ACS reagent) and several times washed with Milli-Q water (total volume used 10 L) until constant pH value (3–4) was achieved.

Synthesis of silver nanoparticles

100 mL of $AgNO_3$ 0.001 M was placed in a reaction vessel. Under magnetic stirring, 10 mL of deionized water containing 0.01 g of gallic acid was added to the Ag^+ solution. After the

addition of gallic acid, the pH value of the solution was immediately adjusted to 11 using a 1.0 M solution of NaOH [9].

Characterization of Particle Size

The average particle size and size distribution were determined by quasielastic laser light scattering with a Malvern Zetasizer (NANO-ZS, Malvern Instruments Ltd., Worcestershire, UK). A 1.5 mL distilled water solution of the nanoparticle (1 mg/mL) was put into a polystyrene latex cell and measured at detector angle of 173°, wavelength of 633 nm, refractive index of 0.30, real refractive index of 1.59, and temperature of 25 °C.

Cultivation of bacteria strains

Staphylococcus aureus (NCTC 8511), *Escherichia coli* (NCTC 13216) and MRSA (ST239) were obtained from the Czech Collection of Microorganisms, Faculty of Science, Masaryk University (Brno, Czech Republic). Cultivation media (Luria Bertani) were inoculated with bacterial culture and were cultivated for 24 h on a shaker at 600 rpm and 37 °C.

Measurement of Inhibition Zones

To determine the antimicrobial effect of composites of GO with nanoparticles the measurement of the inhibition zones was performed. Agar surface in Petri dish was covered with a mixture of 60 mL of 24 h culture of *E.coli*, *S. aureus* and MRSA in the exponential phase of growth, and 3 mL of PCA medium (Plate Count Agar). Excess volume of the mixture of the Petri dishes was aspirated. Discs (ϕ 4 mm) were mixed with tested solutions of composites in Eppendorf tubes. Wet discs were then laid crosswise on a Petri dish with two discs per dish. The Petri dishes were insulated against possible external contamination and placed in a thermostat set at 37 °C for 24 h. After 24 h of incubation, the inhibition zones were measured and photographed in each Petri dish.

RESULTS AND DISCUSSION

The SEM micrographs (Figure 1A) confirmed the preservation of the original structure of the large area of GO. The SEM micrographs also enabled determining the degree of exfoliation, which is crucial for nanoparticular character. Elemental analysis of GO showed carbon as the main component (65 %). EA of GO revealed high degree of oxidation with decreasing content of oxygen in GO (32 %). The trace of sulphur was detected as the residue after the reaction of graphite with sulfuric acid (0.4 %). The FTIR analysis (Figure 1B) showed the presence of carbonyl, hydroxyl and epoxy groups on the GO surface. Measuring inhibition zones was the test to determine the antimicrobial properties of the compounds of interest. The results were obtained after 24 h long cultivation. Silver nanoparticles led to the significant increase in the inhibition of growth of culture of *E.coli*, *S. aureus* and MRSA. Size of inhibition zones (Figure 1E, 1F, 1G) for silver nanoparticles was about 6 mm for *E.coli* and *S. aureus*. The smallest zones of inhibition were determined for MRSA (5 mm). The nature and size of prepared particles was verified by Dynamic Light-Scattering (DLS) (Figure 1C), directly in the solution, without the drying step.

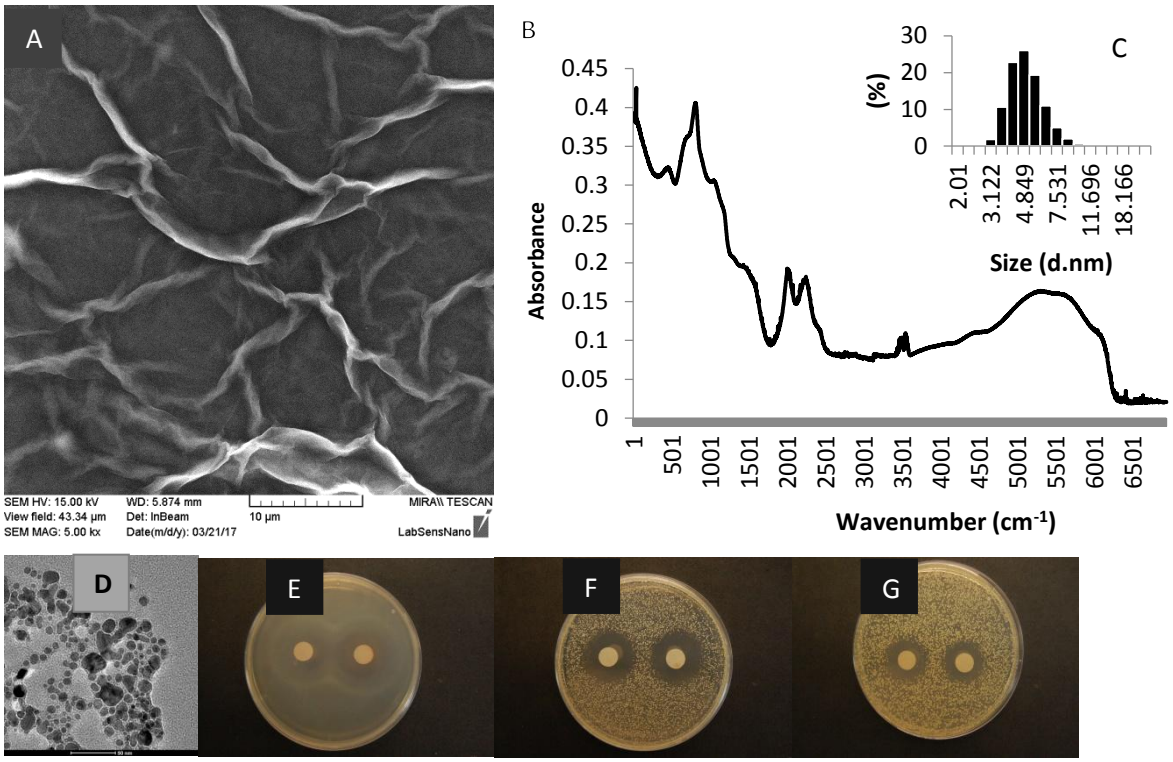

Figure 1.: (A) SEM image of GO; **(B)** ATR-FTIR spectrum of GO; **(C)** Dynamic light-scattering (DLS) spectrum of silver nanoparticles dispersions—size distribution by number**:**; (**D)** TEM image of Ag nanoparticles; **(E)**, **(F)**, **(G)** photos of inhibition zones of the highest applied concentrations of silver nanoparticles on *S. aureus*, *E. coli and* MRSA.

CONCLUSION

In this study, we presented our results for composites of graphene oxide with silver nanoparticles and their effect on bacterial strains, such as antimicrobial substances. The composite showed inhibitory effect on three selected bacterial strains (*S. aureus; E. coli*; MRSA).

ACKNOWLEDGEMENT

This research has been financially supported by the Ministry of Education, Youth and Sports of the Czech Republic under the project CEITEC 2020 (LQ1601).

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CHARACTERISATION OF ENTEROKINASE BY CHRONOPOTENTIOMETRIC STRIPPING ANALYSIS

Martina ČIERNA[1], Miroslav GÁL[1*]

[1] *Department of Inorganic chemistry technology and materials, Faculty ofChemical and food technology, Slovak university of technology in Bratislava, Radlinského 9, 812 37Bratislava, Slovak Republic*
**xcierna@stuba.sk*

Enzymes are one of the most important biomolecules, which act as catalysts by increasing the rate of chemical reactions. They are usually composed of one or more chains of aminoacids. Enterokinase, also known as enteropeptidase, is serine peptidase of the intestinal brush border. It plays an important role as a pancreatic juice activator and converts the inactive form of trypsinogen to active trypsin.

The aim of this study is to distinguish native and denatured form of enterokinase by electrochemical methods. Cyclic voltammetry and chronopotentiometric stripping analysis were used to detect changes of the protein structure. Chronopotentiometric stripping analysis is based on the accumulation of an analyte at the working electrode while current is held constant. Denaturation by application of negative potential and carbamide was performed. The possible aggregation of enterokinase at the electrode surface was avoided by utilization carbamide. Three-electrode system with hanging mercury drop electrode as working electrode, a calomel electrode as reference electrode and platinum wire as counter electrode was used. Britton-Robinson and PBS buffer served as a supporting electrolyte and kept the pH of the solution constant.

According to Paleček's study solid electrodes are suitable for the oxidation of nucleic acids and, on the other side, mercury electrodes are more applicable in the investigation of nucleic acids reduction.

Catalytic hydrogen evolution was observed during application of high negative potential(see Figure 1).

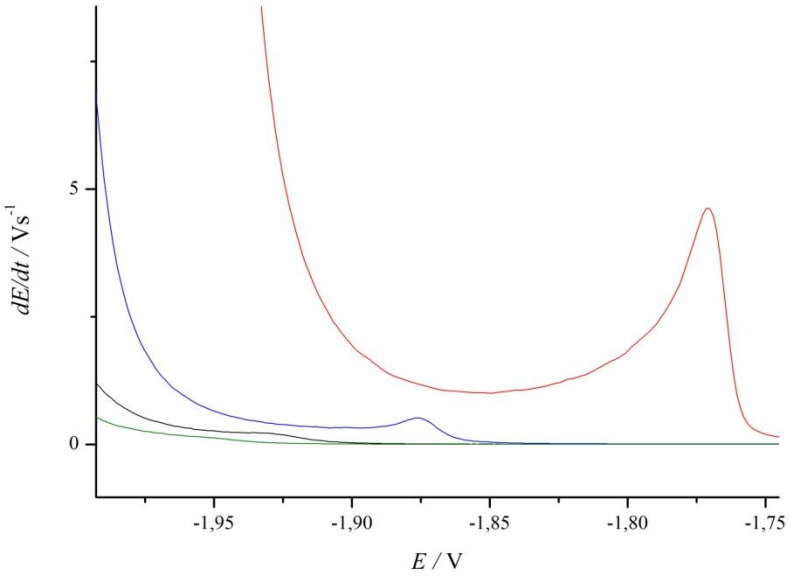

Figure 1. CPS voltammogram of four measurements of 60µl EK in solvent PBS with pH=7,24. Deposition potential -1,4V where current was set to -5µA (red), -25µA (blue), -45µA (black), -65µA (green). Duration time was set to 480s.

Our results have shown that the native form of enterokinase did not provide H peak. In the case of denatured form of enterokinase, H peak was detected. The intensity and position of H peak depends on the applied potential, stripping current and on deposition time. Moreover, the position of reduction signal of a disulfide bonds in protein is affected by the protein structure.

It is known that numerous health concerns are closely connected with the changes of the structures of the respective proteins. Therefore our study can be helpful in clinical practice.

ACKNOWLEDGEMENT

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SURFACE PLASMON RESONANCE BIOSENSOR FOR DETECTION OF PREGNANCY ASSOCIATED PLASMA PROTEIN A2

Erika GEDEONOVÁ[1], Markéta BOCKOVÁ[1], Xue CHADTOVÁ SONG[1], Kateřina LEVOVÁ[2], Marta KALOUSOVÁ[2], Tomáš ZIMA[2], Jiří HOMOLA[1*]

[1] *Institute of Photonics and Electronics, The Czech Academy of Sciences, Czech Republic*

[2] *Institute of Medical Biochemistry and Laboratory Diagnostics of Charles University - the First Faculty of Medicine, and the General University Hospital, Czech Republic*

**homola@ufe.cz*

Pregnancy associated plasma protein A2 (PAPP-A2) is a metalloproteinase from metzincin family that plays multiple roles in fetal development and post-natal growth and it is believed to be connected to disturbances during pregnancy as well as other disorders [1].

In this work we present the development of a novel surface plasmon resonance (SPR) based assay for the detection of PAPP-A2 in blood samples. The SPR biosensor utilizes a sandwich assay with functionalized gold nanoparticles for signal enhancement. This combination meets the requirements arising from the complexity of blood matrix and provides sufficiently low detection limit (3.6 ng/ml) required for analysis of clinical samples [2].

Furthermore, we demonstrate the use of this assay for the detection of PAPP-A2 in clinical samples. The clinical samples include blood serum from two groups of donors: pregnant women and a control group consisting of healthy non-pregnant individuals. We confirmed that the average value of PAPP-A2 in blood serum of pregnant women was increased by more than two orders of magnitude in comparison with the control group.

To ascertain the potential function of PAPP-A2 in pathology of other (pregnancy unrelated) diseases, further research needs to be performed. Currently we pursue integration of SPR and electrochemical (EC) techniques to simultaneously provide both, electrochemical and SPR information about the system under study. We anticipate that this tandem EC-SPR biosensor will yield better understanding of involved molecular processes.

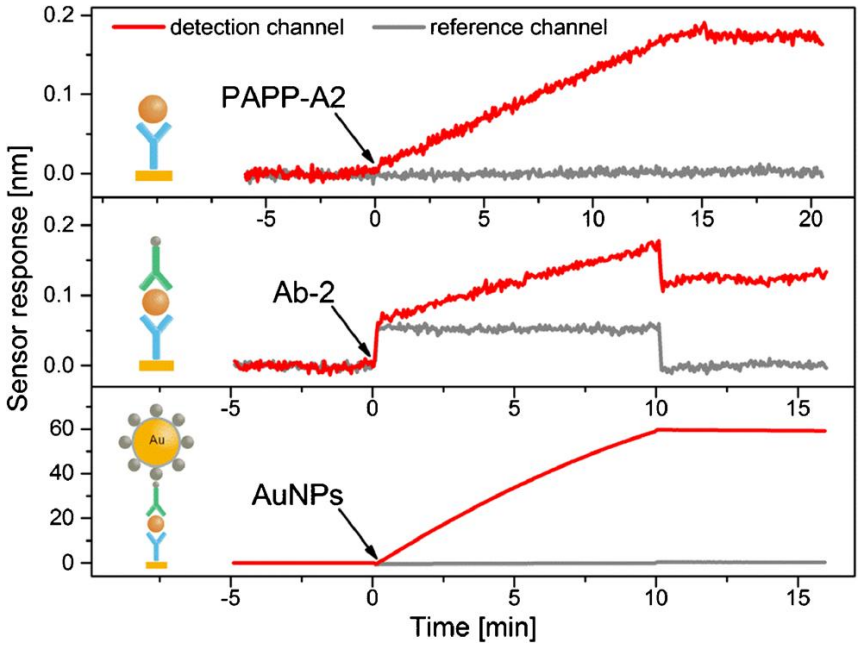

Figure: The sensor response corresponding to respective steps of the assay for detection of PAPP-A2 in buffer. Direct detection of PAPP-A2 (top), binding of biotinylated Ab-2 (middle), binding of streptavidin-coated AuNPs (bottom)

ACKNOWLEDGEMENT

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ELECTROCHEMICAL INVESTIGATION OF NEW LOW-TEMPERATURE ELECTROLYTES FOR ALUMINIUM PRODUCTION

Emília KUBIŇÁKOVÁ*, Ján HÍVEŠ, Vladimír DANIELIK

Institute of Inorganic Chemistry, Technology and Materials, Faculty of Chemical and Food Technology STU in Bratislava, Radlinského 9, 812 37 Bratislava, Slovak Republic

*emilia.kubinakova@stuba.sk

The traditional electrolyte for electrochemical preparation of aluminium is composed of molten cryolite and alumina (2-5) wt %, as electroactive material. Melts are modified by addition of aluminium fluoride (5-12) wt %, calcium fluoride (still present as an impurity up to 5 wt %) and also by other fluoride additives (LiF, MgF_2). This technology uses carbon electrodes to decompose alumina dissolved in cryolite. Reaction products are liquid aluminium and CO_2. The process is operated at temperatures from 950 °C to 970 °C, which is energetically very demanding. One of the objectives of the industry research is to lower the operating temperature of the electrolytic process. The benefits evoked of a temperature decrease in the aluminium potlines are for example: reduction of the energetic consumption, increase of the cell life (decrease of corrosion rates), reduction of production costs, improving of the global environmental footprint, etc.. Low-temperature electrolytes are related to the new "inert" electrode materials. Primary product evolving on the electrode by using the dimensionally stable anodes and wettable cathodes would be oxygen instead of carbon dioxide [1]. Physicochemical properties of low melting melts were investigated only in a lesser extent [2-4].

Low-temperature electrolytes with higher additions of aluminium fluoride have different properties as industrial electrolytes used. So, it is of most importance to study their physicochemical properties. Electrical conductivity is very important bath characteristic for aluminium electrolysis. The main difficulty in determining electrical conductivity of molten fluoride mixtures is to select a suitable material for the conductance cell.

Electrochemical impedance spectroscopy (EIS) was used as a measuring technique. The electrical conductivity of various multi-component cryolite-based mixtures was studied in tube-type cell consisting of graphite crucible and electrode made of pyrolytic boron nitride tube with tungsten rod, detail description in paper [5]. Position of measury electrodes was fixed. *Ac*-techniques were used with a sine wave signal with small amplitude (10 mV) in the high frequency range. The Solartron Impedance/Gain-Phase Analyzer 1260 and Solartron ECHI 1287 instruments were used for the measurements of cell impedance at variable frequency (from 100 Hz to 100 kHz). Measured impedance data were evaluated using by nonlinear regression analysis according to equivalent circuit. Obtained electrolyte resistance was then used for calculation of the electrical conductivity.

The low-temperature cryolite-based electrolytes with higher addition of AlF_3 (up to 45 mol %) were studied. Molar cryolite ratio MR ($n_{(NaF)}$ / $n_{(AlF_3)}$) varied from 2.0 to 1.2 with step 0.2. The temperature range was (700-1040) °C. Influence of additives as alumina, aluminium fluoride, calcium fluoride, magnesium fluoride and lithium fluoride on electrical conductivity was investigated as a function of temperature.

The basic binary system NaF-AlF_3, individual three component systems, and also multicomponent molten mixtures (four and five component electrolytes (Figure)) were

investigated. The influence of all additions, except of LiF causes decrease in electrical conductivity. The rate of decline is highly depend on the excess of AlF_3 (different MR) and on the temperature. The negative effect on electrical conductivity was negligible in some three-component systems (addition of CaF_2 and MgF_2) at MR=1.6 and 1.8. Influences of these compounds were more significant in multicomponent mixtures. The highest impact on the decline in electrical conductivity has alumina. Lithium fluoride increases the value of electrical conductivity but only at a lesser extent.

The concentration and temperature dependence of the electrical conductivity for all studied low-temperature multi-component systems was described by regression equations.

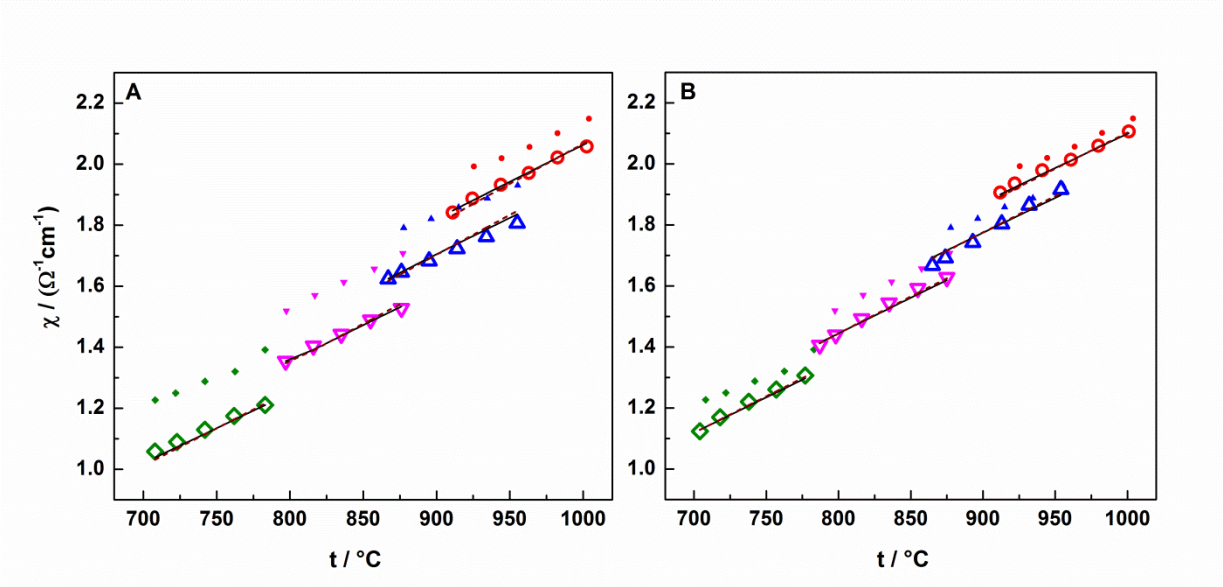

Figure: Electrical conductivity of NaF-AlF_3-Al_2O_3(2%)-CaF_2(5%) (**A**) and NaF-AlF_3-Al_2O_3-CaF_2-LiF(3%) (**B**) systems as a function of temperature for compositions ●/○ MR=1.8; ▲/Δ MR=1.6; ▼/▽ MR=1.4; ♦/◊ MR=1.2. Small full symbols: experimental data for the binary system NaF-AlF_3, larger open symbols experimental data for multicomponent system, full and dotted lines represents data calculated by assembled regression equations.

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STABILITY OF ENCAPSULATED CISPLATIN IN LIPOSOMES- NEW PERSPECTIVE OF CANCER TREATMENT

Petra LANÍKOVÁ[1], Ludmila KREJČOVÁ[2], David HYNEK[2] Vojtěch ADAM[*2,3]

[1]*Faculty of Electrical Engineering and Communication, Brno University of Technology, Technická 10, 616 00, Brno, Czech republic*

[2]*Department of Chemistry and Biochemistry, Faculty of Agronomy, Mendel University in Brno, Zemedelska 1, 613 00 Brno, Czech Republic*

[3]*Central European Institute of Technology, Brno University of Technology, Purkyňova 123, 612 00 Brno, Czech Republic*

**vojtech.adam@mendelu.cz*

Currently, cancer is the one of leading causes of death worldwide. Despite huge progress achieved in cancer treatment during few last decades, the current therapies have their own limitations. Side effects are the most crucial. With the aim to reduce them new strategies in the drug delivery and carriers are searched.

In this study liposomal encapsulation of cisplatin was targeted. Three various liposomes were employed. First type was prepared from 1,2-dioleoyl-*sn*-glycero-3-phospho-*rac*-sodium salt, phosphatidylcholine and cholesterol and it is called L8, second from1,2-dioleoyl-*sn*-glycero-3-phospho-*rac*-sodium salt and cholesterol and it is called L10 and the last one from 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine, phosphatidylcholine and cholesterol and it is called L15. Efficiency of cisplatin encapsulation and stability of cisplatin in liposomes were estimated by voltammetry. With the aim to evaluate stability of liposomes shape and size dynamic light scattering (DLS) was employed. Cisplatin was encapsulated into three variously prepared liposomes.

Platinum based cytostatics are synthetically prepared cytostatics, which interferes with DNA replication. These medicaments are also accompanied with side effects such as nausea, neurotoxicity, nefrotoxicity and haemolytic anemia [1]. Liposomes are possible carriers of drugs because of their small size and capability to reduce side effects. Cisplatin have to be injected intravenously into the human body and this is the reason of side effects, but when is cisplatin encapsulated in liposomes, cisplatin possibly unleash in the moment when it gets to the place of cancer cells [2]. This is an ideal case, but there is still big problem with stability of these liposomes. We use an electrochemical measurement for measuring amount of cisplatin encapsulated in liposomes. The advantage of electrochemical methods is their high sensitivity, selectivity, possibility of miniaturization [3].

Amount of encapsulated cisplatin was measured in three types of liposomes labelled L8, L10 and L15. After preparation of liposomes we obtained lipid film containing 10 mg of liposomes. Liposomes were disssolved in ethylether and next a cisplatin in 0.9% NaCl was added. The ratio of ether phase and aqueous phase was 3:1. In the next step samples were vortexed in order to increase encapsulation efficiency. Next ethyl ether was evaporated and liposomes were extruded to the filtration through membranes with 100 nm pore size. Unencapsulated cisplatin was eliminated by centrifugation in 10 °C for 120 min [4].

Amount of encapsulated cisplatin had to be 3 μg in 1 ml [5]. It was discovered, that in 10 mg liposomes we can encapsulate about 11 μg of cisplatin, which is about three times of therapeutic dose, so we can divide this amount into three therapeutic doses. Each type of liposome had different capability of encapsulation of cisplatin.

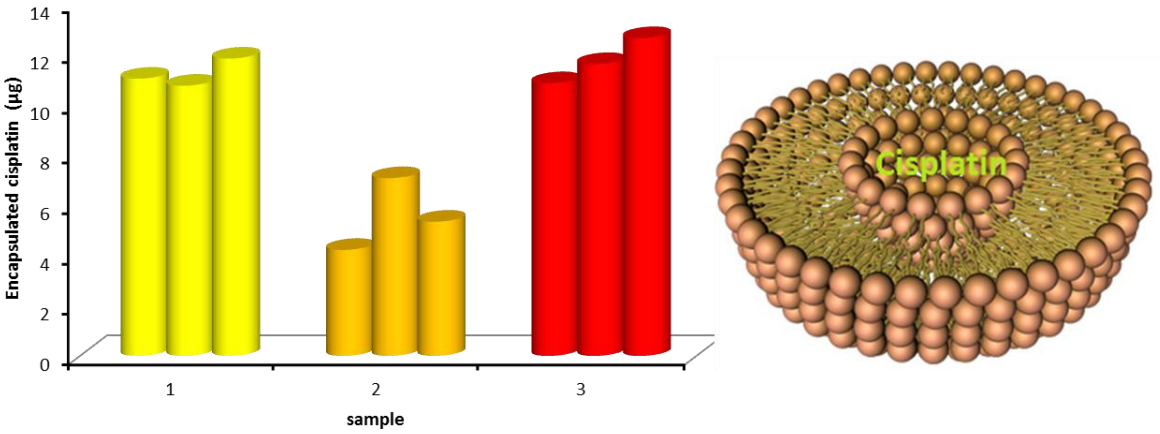

Figure 1: Amount of encapsulated cisplatin in liposomes type L8 (yellow), type L15 (orange) and type L10 (red)

Liposomes have stable size and are capable of encapsulation certain amount of cisplatin. Liposomes called L8 and L10 have the best qualities. These types of liposomes are stable in time and are capable to encapsulate therapeutic dose of cisplatin. L8 also contains cholesterol, which helps protect liposome from destruction in aggressive body fluids [6]. In the next part of research will be tested leakage of cisplatin from all types of liposomes in time by voltammetry.

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ORGANIC ELECTROCHEMICAL TRANSISTORS FOR REAL-TIME CELL OBSERVATION

Lukáš OMASTA[1*], Ota SALYK[1], Jan VÍTEČEK[2,3], Stanislav STŘÍTESKÝ[1], Eva ŠAFAŘÍKOVÁ[2], Michal HRABAL[1], Martin VALA[1], Martin WEITER[1], Lukáš KUBALA[2,3]

1 *Materials Research Centre, Brno University of Technology, Purkyňova 118, 612 00 Brno, Czech Republic*

2 *Institute of Biophysics AS CR, Kralovopolská 135, 612 65 Brno, Czech Republic*

3 *International Clinical Research Center, Center of Biomolecular and Cell Engineering, St. Anne's University Hospital Brno, Pekařská 53, 656 91 Brno, Czech Republic*

**xcomastal@fch.vutbr.cz*

The organic electrochemical transistor (OECT) has a potential for development of innovative biomedical devices. The straightforward fabrication, low-cost printing techniques, individually functionalized and directly interfaced is an advantage of printed OECT. The cultivation of cells in vitro is potentially powerful tool for investigation of drug interaction with the organism for treatment and toxicity testing.

In our work the microplate with multielectrode array of 96 (OECTs) based on semiconductive polymer PEDOT:PSS was developed and fabricated by the screen printing method.

The results of simulation regime without biomaterial but with electrolyte bring the setup information of the experimental conditions. The transconductance g = 1.4 mS was achieved in wide range of gate voltage $V_G = \pm 0.4$ V) when the drain potential $V_D = -0.75$ V was set and the long term relaxation was compensated. The device was tested on 3T3 fibroblasts cell culture and the sudden environmental changes were recorded.

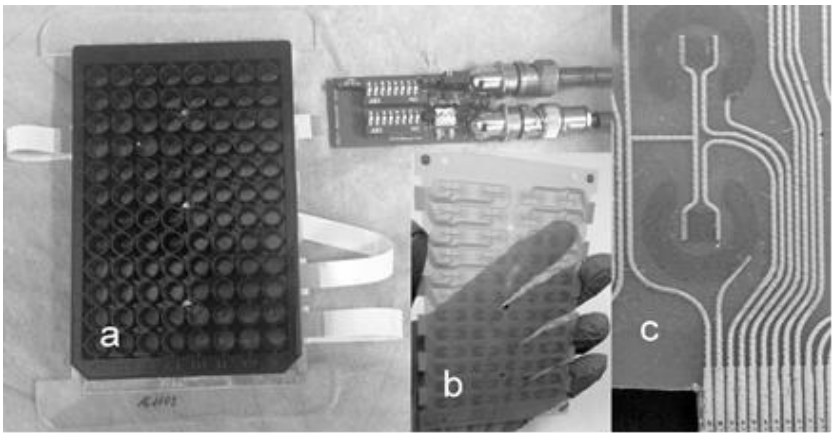

Figure: a) Encapsulated OECT 96 well microplate for electrogenic cells cultivation and investigation with power source and signal amplifier b) foil with the all screen printed OECT array c) detail of the OECT with PEDOT:PSS printed channel and gate electrode.

ACKNOWLEDGEMENT

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ELECTROCHEMICAL DETECTION OF DNA METHYLATION USING MULTIWALLED CARBON NANOTUBES

Jakub PETRULA[1], Zuzana KOUDELKOVÁ[2], Ludmila KREJČOVÁ[2], Lukáš RICHTERA[2], David HYNEK[2], Vojtěch ADAM*[2,3]

[1] *Faculty of Electrical Engineering and Communication, Brno University of Technology, Technická 10, 616 00, Brno, Czech republic*

[2] *Department of Chemistry and Biochemistry, Faculty of Agronomy, Mendel University in Brno, Zemědělská 1, 613 00 Brno, Czech Republic*

[3] *Central European Institute of Technology, Brno University of Technology, Purkyňova 123, 612 00 Brno, Czech Republic*

vojtech.adam@mendelu.cz

DNA methylation is an important epigenetic mechanism which hugely participates in many biological events, such as embryogenesis, cellular differentiation, genetic imprinting and gene expression.[1][2] This work describes approach for the detection and analysis of DNA base and its methylated status, respectively. The presented method is based on the direct electrochemical detection due to electrocatalyc oxidation behaviours of purine and pyrimidine bases at a glassy carbon electrode modified with multiwalled carbon nanotubes (MWNTs/GCE) film. As a result, the modified MWNTs/GCE was successfully applied to the evaluation of all DNA bases including guanine (G), adenine (A), cytosine (C), thymine (T) and 5-mC. The part of the experiment is also a construction of biosensor for indirect DNA base detection. The biosensor is based on the immobilisation of specific DNA sequence on the surface of oxidised MWNTs and detection of direct oxidation signals of bases. Prior to detection, the optimised parameters from the first approach will be considered here as well.[9]

In mammals, DNA methylation occurs almost exclusively in region of CpG dinucleotides, where DNA methyltransferases catalyse the transfer of a methyl group from S-adenosylmethionine to the position 5 of methylcytosine.[3] This covalent modification of DNA plays a very essential role in transcriptional silencing of tumor suppressor genes, what is always associated with diseases. Hypermethylation status of CpG islands can lead to human carcinomas, such as pancreas, lung, leukemia and prostate tumors. Therefore, the detection and analysis of methylation status of DNA can provide a powerful tool for understanding molecular pathology for early cancer diagnosis, give insights into the mechanism of gene repression, and eventually lay the foundation for developing a new cancer treating drugs.[4][5]

Electrochemical methods have unique advantages for detection of DNA due to their rapidity, convenience and ease of miniaturization for small-volume samples. If C and 5-mC can be oxidised with enough potential difference, there is a solid chance to distinguish DNA methylation without any pretreatment.[7] However, there is a challenge to overcome, which is a difficulty of pyrimidine oxidation due to relatively positive oxidation potential, which requires electrode materials having a wide potential window and high electro-activity. This can be solved by using MWNTs, which has unique chemical, electronic and mechanical properties. Therefore, DNA bases can be directly oxidized electrochemically by using MWNTs/GCE modification. [8]

Here we tried to establish a sensitive sensor to detect DNA methylation using MWNTs. MWNTs modified electrode has a large effective surface, wide potential window and excellent electronic properties. Following the principles of DNA oxidation, this method has been successfully applied to the DNA methylation detection. The MWNTs/GCE provides an excellent electrochemical activity towards the oxidation of DNA bases due to the advantages of wide potential window and an enlarged electrode surface. As a result, all purine and pyrimidine bases and 5-methylcytosine (5-mC) electrochemical signals were clearly obtained and exhibited well defined oxidation peaks. Another great challenge was encountered due to the interference from thymine (T) which has almost the similar oxidation potential as that of 5-mC thus, the visual recognition is almost impossible. In order to eliminate the interference and recognise the oxidation peaks of those bases, we provided a couple of measurements involving thymine, 5-mC and its mixture, respectively. As a result, necessary information such as peak position and half-width were obtained and the approach of curve fitting was used to mathematically distinguish overlapping peaks.[9]

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THE ORIGIN OF GUANINE OXIDATION DOUBLE PEAK

Aneta VEČEŘOVÁ, Libuše TRNKOVÁ, Iveta TŘÍSKOVÁ
Masaryk University, Faculty of Science, Department of Chemistry, Kamenice 5, CZ-62500 Brno, CZ

Cyclic voltammetry is very favourite method for research of nucleic acids because voltammetry provides not only information about their electrochemical redox activity but also about their composition and secondary, respectively tertiary structures. Therefore, voltammetry can be used for their structural analysis. Using mercury electrodes (SMDE - static mercury electrode or HMDE - hanging mercury electrode), many possible conformation changes of nucleic acids are projected into the form of reduction and/or oxidation peaks whose analysis can offer valuable information. One of these peaks is guanine oxidation signal which appears after previous guanine reduction at negative potentials close to hydrogen evolution [1]–[3] and may have the form of single or double peak (Figure; A). Interestingly, the origin of guanine oxidation double peak (GODP) is unknown.

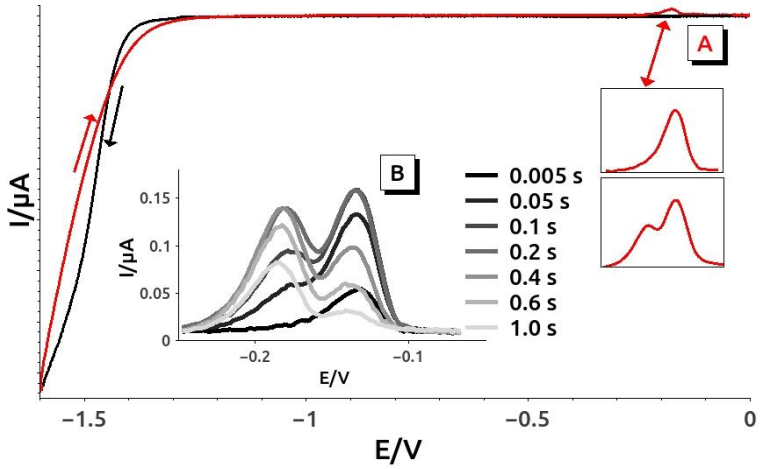

Figure: A) Cyclic voltammogram at SMDE with image of two possible shapes of guanine oxidation signals; B) the effect of time of reduction (at -1.45 V) on the shape of GODP; sample d(GGG), concentration 0.72 µM, acetate buffer, pH 4.5, scan rate 0.9 V/s, step 2 mV, in CV vertex potential -1.6 V.

Our research, aimed at the explanation of GODP origin, was based on earlier findings that the double peak shape depends on scan rate and/or vertex potential [4]–[6]; therefore, time of reduction may play a significant role in redox processes of guanine moieties in oligo- and polynucleotides. It was newly revealed that two distinct oxidation peaks probably appear as an consequence of guanine oxidation in two different adsorption conformations of DNA which are controlled by both time (Figure, B) and potential of reduction.

The results of this study form the starting point not only for further studies of redox processes of DNA and RNA fragments on solid electrodes but also for development of a low cost and rapid indicator of their structural properties.

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DETERMINATION AND COMPARISION OF MICELLE RADIUS OF DIFFERENT SURFACTANTS USING FÖRSTER RESONANCE ENERGY TRANSFER

Zuzana ADAMCOVÁ[1,2*], Filip MRAVEC[1,2], Miloslav PEKAŘ[1,2]

[1] *Institute of Physical and Applied Chemistry, Faculty of Chemistry, Brno University of Technology, Purkyňova 464/118, 612 00 Brno, Czech republic*

[2] *Materials Research Centre, Faculty of Chemistry, Brno University of Technology, Purkyňova 464/118, 612 00 Brno, Czech republic*

**xcadamcovaz@fch.vut.cz*

This contribution is focused on utilization of Förster resonance energy transfer as a nanoscale ruler[1] in order to determine micellar radii of three cationic surfactants with different hydrophobic chain length – CTAB (C_{16}), Septonex (C_{15}) and TTAB (C_{14}). As a convenient donor-acceptor pair, Perylene (which is solubilized inside micelles) and Fluorescein (situated in the outer hydrophilic part of micelles) were chosen. Located in close proximity (nm), Fluorescein quenches fluorescence of Perylene, the effectivity of which is in direct relation to their distance[2] – radius of micelle in this case. Results obtained by FRET were compared with FCS (Fluorescence Correlation Spectroscopy) measurements, which determine micelle radius on the basis of its diffusion coefficient. In this experiment micelles were labeled only with Perylene in order to avoid effects of fluorescent labeling on the surface of micelles.

According to results presented in the Table, there is correlation between number of carbons in hydrophobic chain of the surfactant molecule and size of corresponding micelle, as decrese of micelle radius with decreasing hydrophobic chain length was observed in both experiments. Slight differences in particular values might be due to determination of *hydrodynamic* radius in case of FCS or location of Perylene not exactly in the centre of the hydrophobic core of micelles.

Table : Comparision of micellar radii of three different surfactans determined by FRET and FCS

Surfactant	Radius FRET [nm]	Radius FCS [nm]
CTAB	3.71 ± 0.01	4.56 ± 0.07
Septonex	3.64 ± 0.01	3.42 ± 0.01
TTAB	3.54 ± 0.03	3.16 ± 0.01

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FLUORESCENE METHOD FOR MEASURING CELL PROLIFERATION BY IMAGE ANALYSIS OF CELL CONFLUENCY

Larisa BAIAZITOVA[1*], Josef SKOPALÍK[1], Ondřej SVOBODA[1], Jiří CHMELÍK[1], Ivo PROVAZNÍK

1 *Department of Biomedical Engineering, Faculty of Electrical Engineering and Communication, Brno University of Technology, Technická 3082/12, 616 00 Brno, Czech Republic*
*xbaiaz00@stud.feec.vutbr.cz

Assessing of cell viability is the key steps of cell cultivation. Counting of cells number per cm^2 and measuring cell confluency are another two important characteristics, since it is important to know when cells have to reach their optimum density. For example, to study of cell migration applied chemoattractant or growth factor we need to analyze rates of cell proliferation and motility. It is a more complicated in transwell migration assay, were cell monolayer generally cultivated at microporous membrane. In this instance we can used fluorescence life cell imaging. For qualitative analysis algorithms of image segmentation are applied. In this paper an example of segmentation of fibroblasts (3T3 Cells Line) with different levels of confluency is demonstrated.

Fibroblasts was cultured in sterile in vitro chamber in a humidified incubator with 5% CO_2 atmosphere at 37°C. The Dulbecco Modified Eagle's Medium (GIBCO, 11960044) supplemented with 2mM L-Glutamine (Thermofisher Scientific, 21051040) and 2% Penicilin/Streptomycin (Sigma Aldrich, P4333-20ML) was used and exchanged every 2 days until the cells need to split. Cells were passaged when they reached 80%-confluency using 0.25% trypsin EDTA (Sigma-Aldrich, T4049). For our experiment it were seeded on 8 well chamber slide (Lab-Tek, 177445) in density 1000 cells/cm^2. For monitoring of cell proliferation we chose Calcein AM (Sigma-Aldrich, 56496) fluorescence probe because it is stable cytoplasmic stain which is suitable for long-term research. Before scanning cell samples were stained by Calcein AM (concentration 1%) during 10 min. During this time, the Calcein AM permeable through cell membrane, then intracellular esterases cleave the acetoxymethyl (AM) ester group, yielding the membrane-impermeable Calcein fluorescent dye. This is advantageous, because cells with damage membrane don't retain this dye.

Experiments were performed on the confocal laser scanning microscope Leica TCS SP8 X. The picosecond White Light Laser (WLL) Leica Microsystems freely tunable in the spectral range 470-670 nm with 80 MHz repetition rate was used for excitation. The measurement setup was equipped with transparent incubation chamber with temperature and CO_2 level control. The scan was provided with 20X magnification objective. The physical length of image sequence is 581.25 m × 581.25 m with spatial resolution of 512 × 512 pixels. The z-distance between stacks is 0.3 m. The excitation wavelength of 490 nm and emission filter 500-550 nm was set according to the Calcein AM spectrum.

Analyzes of cell confluency are provided by suggested algorithm. We used Matlab computing environment to implement the developed method for the image processing and segmentation of the fluorescence images. First, the z-stacks were summed to avoid errors due to the possible tilt of the sample. Then cells were distinguished from background by segmentation method based on simple thresholding with defined level which is obtained by histogram analysis. Median filter was applied to eliminate individual pixels which are incorrectly identified as cell's pixels. In the last step the cell confluency (in percentages) was calculated

as an area ratio of white to black pixels. Same examples of segmentation can be seen in the Figure.

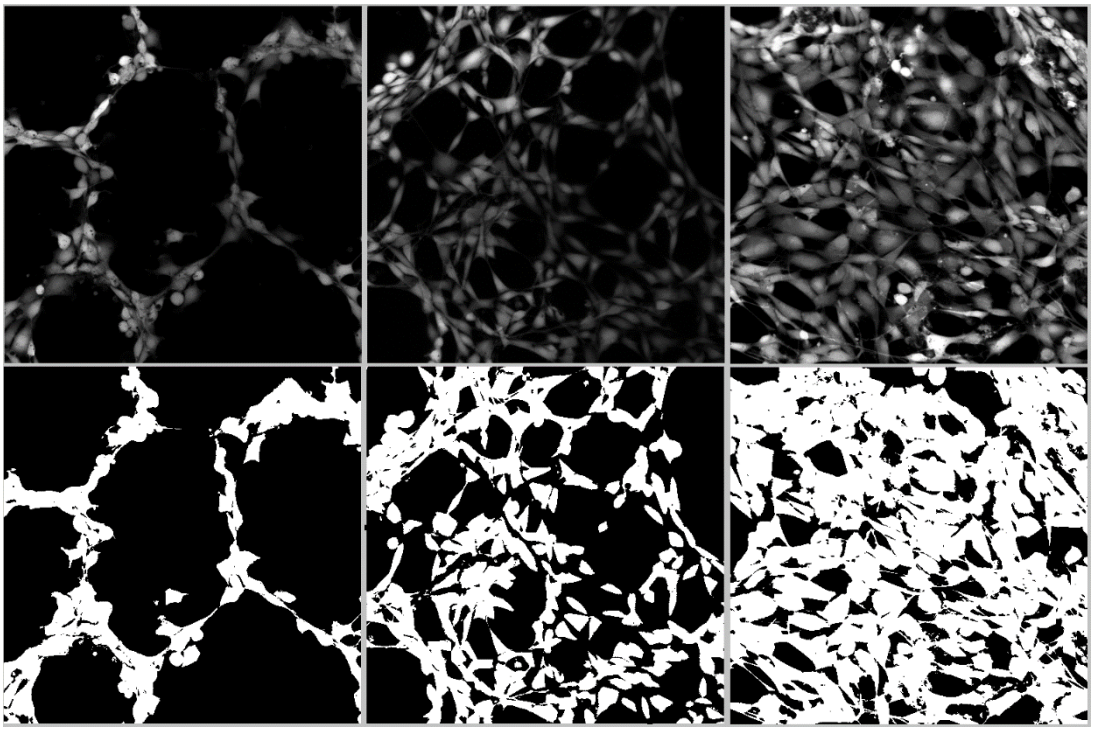

Figure: 3T3 Cell Line proliferation. In the top images are sum of original fluorescence z-stacks images in different confluency (18%, 42% and 78% from left to right). In the bottom images are segmented cells by suggested algorithm.

The proposed algorithm of analysis of cell confluency can be applied to modified culture chambers and different adherent cell lines. We have tested three different cultivating chamber with different coating. Precise cell enumeration and viability computing can help to optimize coating and culture protocol and to improve quality of migration or perfusion assay experiments.

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TITANIUM DIBORIDE AS NEW ELECTRODE MATERIAL FOR ELECTROCHEMICAL PREPARATION OF ALUMINIUM

Michaela BENKÖOVÁ*, Emília KUBIŇÁKOVÁ, Ján HÍVEŠ

Institute of Inorganic Chemistry, Technology and Materials, Faculty of Chemical and Food Technology STU in Bratislava, Radlinského 9, 812 37 Bratislava, Slovak Republic

xbenkoovam@is.stuba.sk

Aluminium is produced electrochemically by the Hall-Héroult process. The traditional electrolyte for electrolysis is composed of molten sodium cryolite (Na_3AlF_6) and alumina (Al_2O_3). Melts are modified by addition of aluminium fluoride (AlF_3), calcium fluoride (CaF_2) and also by other fluoride additives, such as lithium fluoride or magnesium fluoride (LiF, MgF_2). This technology still uses carbon anodes and a liquid aluminium cathode (located on the carbon blocks at the bottom of electrolyser) to decompose alumina dissolved in cryolite. Reaction products are liquid aluminium and carbon dioxide [1]. The process is operated at temperatures of around 960 °C, which is energetically very demanding. The main objectives of the industry are to change the electrode materials and to lower the operating temperature of the electrolytic process. The benefits evoked of a these changes in the aluminium potlines are multiple, such as reduction of the energetic consumption, increase of the cell life (decrease of corrosion rates), reduction of production costs, improving of the global environmental footprint, etc. [2]. Development of the suitable materials for new nonconsumable electrodes is very important. Using by these dimensionally stable anodes, the primary product evolving on the electrode would be oxygen instead of carbon dioxide. Moreover, "inert" electrode materials are related to the new low-temperature electrolytes [3,4].

In recent decades, new inert electrode materials are studied for aluminium production. Different kinds of materials were investigated mostly for inert anodes, in lesser extent for inert cathodes [1]. The most suitable cathode material seems to be titanium diboride [5]. The main advantage of TiB_2 is excellent wettability by molten aluminium. Object of this study is wettability of TiB_2 electrode material at low-temperature range, up to 900 °C. The aim of the work was determination of wettability for materials with various surface roughnesses. Hot-press method was used for preparation of titanium diboride samples. Sessile drop technique was applied for designation of contact angle between the drop of molten aluminium and the surface of TiB_2. The results showed that the wettability of studied materials were very high, close to the perfectly wettable surfaces behaviour even at a lower temperatures.

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PENCIL GRAPHITE ELECTRODE AS A PROMISING TOOL FOR ELECTROANALYSIS OF METHYLGUANINES

Markéta BOSÁKOVÁ, Iveta TŘÍSKOVÁ, Libuše TRNKOVÁ*

Department of Chemistry, Faculty of Science, Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic

libuse@chemi.muni.cz

Nowadays, epigenetics is among the top disciplines of biomedicine. It is actually because epigentic modifications, especially methylation of nucleic acids (NAs), are linked with many diseases and dysfunctions, such as human carcinomas (lung, thyroid, prostate and pancreas), leukemia, etc.[1-4]. Guanine (G), one of the two essential nucleic bases, plays a key role in the DNA oxidation by various types of oxidants and free radicals [5-7]. Although guanine, compared with cytosine, is not a typical recipient of methyl groups, methylated guanines attract the attention as potential drugs and/or markers. Specifically, N7-methyl modification of G is a well-established biomarker for the detection and determination of DNA methylation [8]. Only few examples can be found in the literature regarding to the electrochemical determination of this alkylmodification of G. The electrochemical reduction of 7-methylguanine derivatives (7-methylguanosine and 7-methylguanosine-5‘-phosphate) at the mercury electrode [3, 9, 10] was completed with the study of oxidation behavior of 7-methylguanine at boron-doped diamond electrode and carbon electrodes (glassy carbon electrode and screen printed graphite electrodes)[8]. There are some papers regarding electrochemical behavior of 7N-methylguanine (7N-mG), linked with epigenetic modifications of DNA chain, but there are not any publications regarding other methylated guanine derivatives, such as 1N-, 3N- or 9N-methylguanines (Figure).

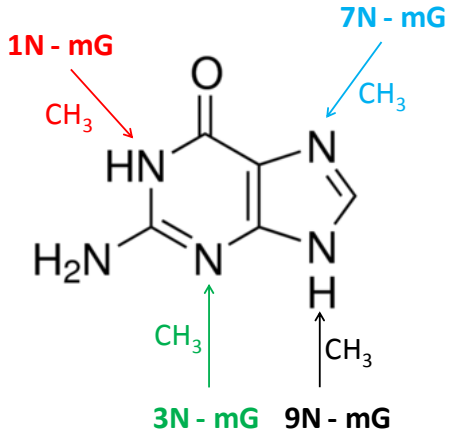

Figure: Guanine and its methylated derivatives

In our research the oxidation processes of methylated guanine derivatives on a polymer pencil graphite electrode (pPeGE) in dependence on pH (phosphate – acetate buffer; pH 3.16 – 7.52) and the position of methyl group in the G molecule (pyrimidine ring vs. imidazole ring) were investigated. It was shown that the polymer pencil leads are an excellent electrode material for the sensitive analysis of N-mGs without the need for the electrochemical pretreatment or other electrode activation procedures[11]. Different oxidation peak heights and potentials of N-mGs demonstrate the effect of of methylation on their oxidation processes. It was found that their oxidation is easier in the case of CH_3- substitution at the pyrimidine ring than at imidazole one.

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LABEL-FREE ELECTROCHEMICAL APTASENSOR FOR CANCER DIAGNOSTIC

Marianna ELIÁŠOVÁ SOHOVÁ[1*], Lenka BABELOVÁ[1,2**], Alexandra POTURNAYOVÁ[1,2] and Tibor HIANIK[1]

[1] *Department of Nuclear Physics and Biophysics, Faculty of Mathematics, Physics and Informatics Comenius University, Mlynská dolina F1, 842 48 Bratislava, Slovakia*

[2] *Center of Bioscience, Slovak Academy of Sciences, Dúbravská cesta 9, 840 05 Bratislava, Slovakia*

**Marianna.Sohova@fmph.uniba.sk; **babelova@me.com*

Cancer diseases are very serious and spreading in 21st century. The best prognosis for cancer treatment is prevention and fast diagnostic. Low cost electrochemical aptasensor, using aptamers („chemical antibodies“) instead of the antibodies is great opportunity for it. Here we propose aptasensor with easy preparation and sensitive detection, which can be even increased with suitable nanoparticles. DNA aptamer sgc8c specific for PTK7 (protein tyrosine kinaze 7) [1] was used as receptor. PTK7 is overexpressed in cancer cells [2, 3]. Leukemic Jurkat cell line was tested as a model system. The thiol-modified sgc8c aptamers were immobilized at the surface of gold electrode. Electrochemical impedance spectroscopy (EIS) has been used to analyze the response of the sensor toward addition of Jurkat or control cells. The sensor revealed quite high sensitivity – already 50 cells/mL was possible to recognize. Control measurement with U266 cells, which do not express PTK7, showed non-specific interaction, so the aptamer sgc8c was specific. Sensor regeneration was also performed and the most promising is 25 mM NaOH, which was able to regenerate sensor surface. In the future the detection limit can be improved by using nanoparticles or specific electrochemical active dyes.

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$LiFePO_4$/SULFUR COMPOSITES AS CATHODE MATERIALS FOR HIGH PERFORMANCE Li-S BATTERIES

Andrea STRAKOVÁ FEDORKOVÁ[1], Katarína GAVALIEROVÁ[1], Tomáš KAZDA[2], Ondrej ČECH[2], Pedro GOMEZ-ROMERO[3]

[1]*Institute of Chemistry, Faculty of Science, P.J. Šafárik University, Moyzesova 11, SK-041 54 Košice, Slovakia*

[2]*Department of Electrical and Electronic Technology, Faculty of Electrical Engineering and Communication, Brno University of Technology, Technicka 10, 616 00 Brno, Czech Republic*

[3]*Institut Català de Nanociència i Nanotecnologia, ICN2, CIN2 / Consejo Superior de Investigaciones Científicas (CSIC), Campus UAB, E-08193 Bellaterra, Barcelona, Spain*

*katarina.gavalierova@gmail.com

Abstract

Today, the modern world cannot be described without considering lithium-ion batteries. Current concern about limited energy resources, coupled to the need to decrease greenhouse gas emissions, has brought the need to consider renewable energies at a large scale together with the use of hybrid and electric vehicles. Among the various energy conversion/storage systems proposed over the two last centuries, electrochemical storage and more specifically batteries seem to be very well positioned to satisfy these needs, but research to meet the application requirements is still an imperious need [1].

Lithium-sulfur (Li-S) batteries are regarded as one of the next-generation energy storage systems due to the extremely low-cost sulfur and their high energy density [2-4]. The price of sulfur per metric ton was as low as $160 USD in 2012 [5]. The theoretical capacity of sulfur is 1672 mAh/g (calculated based on $S^0 \leftrightarrow S^{2-}$). Coupled with the average operating voltage of a Li-S cell (2.15 V vs Li^+/Li^0) and the theoretical capacity of a pure lithium anode (3862 mAh/g, calculated based on $Li^+ \leftrightarrow Li^0$), the energy density can be estimated as high as ~2500 Wh/kg, which is an order of magnitude higher than that of traditional Li-ion batteries. Although Li-S batteries possess many advantages, low active material utilization, capacity degradation, self-discharge, poor Coulombic efficiency, poor cycle life, and electrode volume expansion are still the challenges remaining with the Li-S cells [2,6,7].

The objective of this work is to investigate the fundamental chemistry of sulphur composites and lithium polysulfides and develop new functional electrode materials and architectures for high energy, low cost Li-S batteries.

RESULTS AND DISCUSSION

Fig. 1 shows the morphology of S-$LiFePO_4$ and the S-$LiFePO_4$-MWCNTs cathode material. The sulfur is composed of loose agglomerates of 10–20 μm primary particles. During the mechano-chemical reaction, the sulfur particles are covered with the finer $LiFePO_4$ particles on the surface. MWCNTs are distributed very well on the surface of the composite cathode material. This network structure confirms close contact between the conductive carbon, sulfur and MWCNTs, providing not only excellent electron pathways for the insulating sulfur but also many adsorbent points on the surface of MWCNTs to avoid the loss of the soluble polysulfides into the electrolyte.

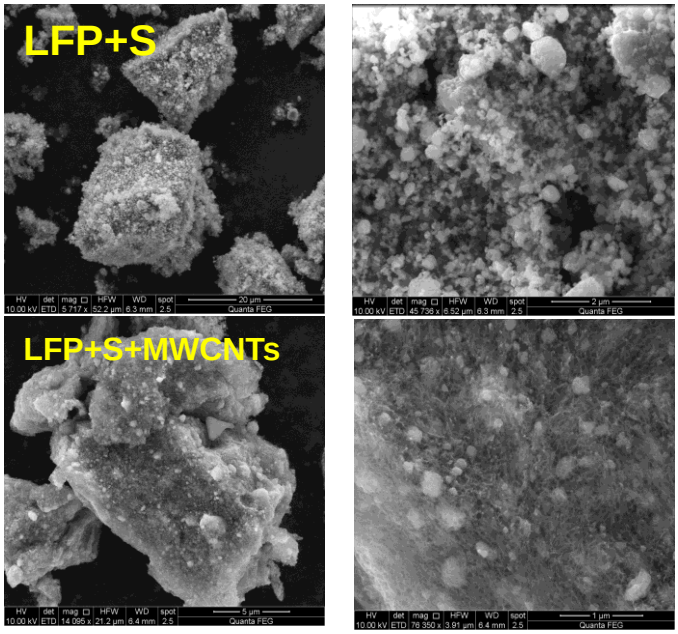

Fig. 1 SEM images showing the morphology of the S-$LiFePO_4$ sample (up) and S-$LiFePO_4$-MWCNTs sample (down).

It can also be noted that the S-LFP-MWCNT cathode presents a stabilized specific capacity of 980 mAh/g-sulfur a 20 % higher than that of S-C-MWCNT (780 mAh/g-sulfur). The discharge capacity gradually decreases as the current rate is raised from 0.1 C to 0.5 C for both of the cells. The higher discharge capacities of 1170, 855 and 750 mAh/g-sulfur were achieved at a current density of 0.1, 0.2, and 0.5 C, respectively for S-LFP composite material.

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EFFECT OF AMPELOPSIN ON BIOTRANSFORMATION ENZYMES

Petr HODEK[1*], Michaela BEBOVÁ[1], Zdislava BOŠTÍKOVÁ[1,] Eva ANZENBACHEROVÁ[2], Marie STIBOROVÁ[1]

[1] *Department of Biochemistry, Faculty of Science, Charles University, Hlavova 2030/8, 128 40 Prague 2, Czech Republic*

2 *Department of Medical Chemistry and Biochemistry, Faculty of Medicine and Dentistry, Palacky University, Hněvotínská 3, 775 15 Olomouc, Czech Republic*

**hodek@natur.cuni.cz*

Ampelopsin (AMP) is a natural flavonoid (see Fig. 1) which occurs in high quantities in plants, e.g. *Ampelopsis grossedentata* and *Hovenia dulcis*. This flavonoid has attracted attention because of its ability to attenuate the symptoms of ethanol intoxication [1]. Recent studies suggest that AMP counteracts EtOH effects *via* antagonizing the acute EtOH-induced potentiation of γ-aminobutyric acid receptors. It cannot be excluded that DHM only masks an EtOH acute intoxication. More importantly, AMP reduces voluntary alcohol drinking as shown in experiments with rats [2]. Thus, AMP will be possibly used by humans as a food supplement. The extensive intake of AMP might be associated with adverse effects on human health since flavonoids are known modulators of enzymes involved in phase I and II of biotransformation, which participate in carcinogen activation and drug metabolism [3]. Hence, it is important to reveal the impact of AMP on cytochromes P450 (phase I enzymes) and sulfotransferases (SULTs) and *N*-acetyl transferases (NATs) (phase II enzymes). In the present study we focused on the induction and inhibition of SULTs and NATs by AMP.

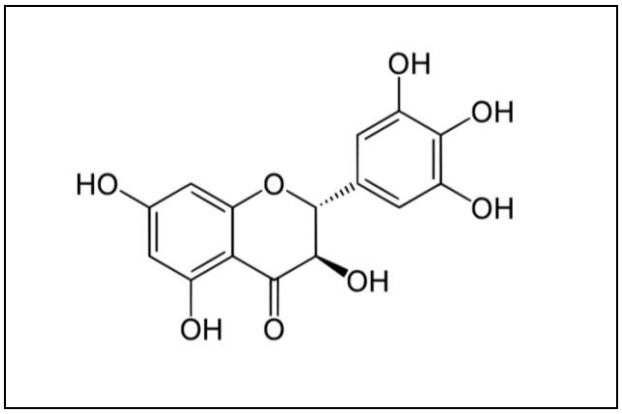

Figure 1: Structure of flavanonol, ampelopsin.

To mimic conditions of human exposure with AMP, all flavonoids tested were administrated *per os* (60 mg/kg b.w.) by gastric gavage to stomach of rats for three consecutive days. Then, cytosolic samples were prepared from liver, small intestines and colon. The activity of SULT1A1, NAT1, and NAT2 in cytosolic samples was determined by colorimetric assay using specific substrates *p*-nitrophenylsulphate, sulfamethazine and 4-aminobenzoic acid, respectively. Protein expression of these enzymes was determined on Western blots using anti-SULT and anti-NAT antibodies. Moreover, the AMP mediated inhibition of SULT and NAT activities was examined with recombinant enzymes.

The animal treatment with AMP induced SULT1A1 activity in all tissues studied with the exception of small intestines. However, no effect of AMP administration on NAT activities was found. These results are in agreement with those from Western blots developed with anti-

SULT and anti-NAT antibodies. Marked increase in SULT1A1 protein expression (compared to un-treated control) was detected in liver cytosol of animals treated with AMP. Next, the AMP inhibition of SULT1A1, NAT1, and NAT2 activities was examined. Data showed that AMP is ineffective towards SULT1A1 specific activity. On the other hand, both NAT1 and NAT2 activities were effectively inhibited by AMP (IC_{50} < 10 μmol/L).

In conclusion the present data suggest only a low potential risk associated with the SULT1A1 induction and NAT1 and NAT2 inhibition at systemic concentrations after the AMP ingestion.

ACKNOWLEDGEMENT

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AGGREGATION BEHAVIOUR OF HYALURONAN-CATIONIC SURFACTANT SYSTEM STUDIED BY FLUORESCENT HYDROPHOBIC PROBES

Petra HOLÍNKOVÁ[1*], Miloslav PEKAŘ[1]

[1] *Institute of Physical and Applied Chemistry, Faculty of Chemistry, Brno University of Technology, Purkyňova 464/118, 612 00 Brno, Czech Republic*

**xcucekajova@fch.vut.cz*

Hyaluronan is a naturally occurring linear polysaccharide, which is common in connective tissues of vertebrates [1]. As a component of extracellular matrix, hyaluronan plays an important role in migration and proliferation of cells, immune response of organism; it facilitates wound healing and acts as a lubricant and shock absorber in the synovial fluid. Also hyaluronan was found to be implicated in tumour cell behaviour [2]. Because many molecules of drugs are hydrophobic, hyaluronan has been chemically modified to induce micelle-like properties or directly conjugated with hydrophobic drugs. But this process could change its biological activity and compatibility. Next way could be the physical interaction with suitable substance which allows solubilisation of hydrophobic molecules in presence of hyaluronan. For example, interactions between hyaluronan and cationic surfactant may lead to formation aggregates in which the surfactant hydrophobic domains can solubilize hydrophobic substances and hyaluronan has a role biocompatible carrier and targeted agent.

This paper deals with study of interaction between hyaluronan and cetyltrimethylammonium bromide (CTAB) in aqueous and physiological solution (0.15 M sodium chloride). In samples, concentration of hyaluronan was constant and concentration of CTAB was increased (hundred to ten times less than its critical micelle concentration in aqueous solution). For study of aggregation in this systems, two hydrophobic fluorescent probes were used – perylene and diphenylhexatriene. The emission spectra, total fluorescence intensity and fluorescence anisotropy (polarized fluorescence) of the samples were measured. It was observed that the initial addition of CTAB to hyaluronan aqueous solution leads to forming some small hydrophobic domains linked to hyaluronan chains. Then an increasing concentration of CTAB causes phase separation and formation of a condensed phase with large amount of hydrophobic domains. In physiological solution, condensed phase is not created and probably free micelles are formed in the solution, possibly mixture of micelle linked to hyaluronan chain with free micelle, are formed in the solution.

ACKNOWLEDGEMENT

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A NOVEL PROCEDURE FOR CONSTRUCTION OF AN APOFERRITIN NANOCARRIER WITH ENCAPSULATED ELLIPTICINE

Radek INDRA[1], Marek WILHELM[1], Zbyněk HEGER[2], Simona DOSTÁLOVÁ[2], Vojtěch ADAM[2], Marie STIBOROVÁ[1*,]

[1] *Department of Biochemistry, Faculty of Science, Charles University, Albertov 2030, 128 40 Prague 2, Czech Republic*

[2] *Department of Chemistry and Biochemistry, Laboratory of Metallomics and Nanotechnology, Mendel University in Brno, Zemedelska 1, 61300 Brno, Czech Republic*

**stiborov@natur.cuni.cz*

Abstract. A novel procedure to construct a nanocarrier from naturally found protein apoferritin encapsulating ellipticine was developed. Ellipticine is able to be encapsulated into apoferritin forming nanoparticles (ApoElli) and at acidic pH reversely released from this form. Further, ApoElli is capable to interact with microsomal subcellular particles resulting in ellipticine release and its subsequent oxidation by microsomal cytochromes P450. This ellipticine-nanoparticle seems to be a promising tool in cancer treatment.

INTRODUCTION

Ellipticine is an efficient anticancer compound that functions through multiple mechanisms of its action [1,2]. Ellipticine was found to inhibit cell growth, to arrest cell cycle, and to induce the mitochondria-dependent apoptotic processes in cells [1,2]. DNA damage is responsible for ellipticine's biological effects. Of them, the formation of covalent DNA adducts after ellipticine enzymatic activation with cytochromes P450 (CYP) and peroxidases, is the major mechanism of its antitumor activities [1,2]. This drug however exhibits also severe adverse toxic effects [2]. Hence, we are aimed to develop efficient and reliable methods for targeted delivery of ellipticine (and/or other anticancer drugs) as well as on preparation of this drug in the forms that exhibit lower side effects and leads to an increase in their anticancer effects. One of the aims is to develop nanocarriers containing this drug. Endogenous particles seem to be most promising for such purposes; especially those involved in the cellular uptake pathways. They are naturally biocompatible and biodegradable and they also provide easy passage through the cell membranes [3]. Apoferritins (APO), which are responsible for the storage and transfer of iron [4], can provide these much needed properties. APO protein subunits assemble to form a hollow cage into which diverse substances, such as drugs, can be placed. It was shown that while disassembled, APO can be mixed with drug molecules and they are encapsulated within APO cavity once reassembled [4]. The aim of this study was to construct a nanocarrier based on apoferittin containing encapsulated ellipticine.

MATERIAL AND METHODS

Ellipticine dissolved in distilled water (1 mg/ml) with addition of 1 M HCl (a ratio of 150:1) was encapsulated into apoferritin as follows: 20 μl of horse spleen apoferritin (50 mg/ml) was mixed with 200 μl of water and 100 μl of ellipticine (15 min). Then 0.66 μl of 1 M NaOH was added to increase the pH and encapsulate ellipticine to apoferritin (15 min). The sample was diafiltrated three times with water using Amicon® Ultra - 0.5 mL 3K, at 13000 g for

5 min. The visualization of nanocarrier prior to removal of released ellipticine was performed using transmission electron microscopy (TEM).

RESULTS AND DISCUSSION

The simple-to-use encapsulation protocol (creating ApoElli) was developed and the prepared nanocarrier was characterized. The nanocarrier exhibits narrow size distribution (Fig. 1) suggesting to be suitable for entrapping of the hydrophobic molecule of ellipticine.

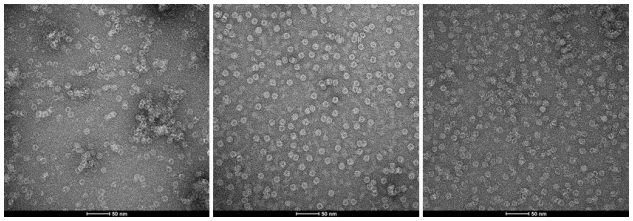

Fig. 2 TEM micrographs of apoferritin (**A**), freshly prepared ApoElli (**B**) and ApoElli stored for 1 month at 4^{o} C (**C**).

Ellipticine is gradually released from its ApoElli form into the water environment under acidic pH; more than 33 % ellipticine was released after 48 hrs incubation at pH 6.5. In contrast, ApoElli is stable after its storage at physiological pH (7.4) up to 1 month at 4 °C; less than 5 % ellipticine was released after this storage. Release of ellipticine from ApoElli nanoparticles was however possible also at physiological pH 7.4, when they were incubated with rat liver microsomes (a cellular membrane fraction of broken endoplasmic reticulum). The presence of these membrane particles accelerates release of ellipticine from ApoElli and makes it possible to be transferred into microsomes even at pH 7.4. Microsomes incubated with free ellipticine and/or its ApoElli nanocarrier in the presence of NADPH (a cofactor of a CYP-mediated enzyme system present in microsomes) were capable of oxidizing ellipticine to its metabolites increasing its anticancer efficiencies, both under pH 6.5 and 7.4. We hypothesized that the microsomal membrane particles may interact with ApoElli, facilitating ellipticine release even at pH 7.4 and its subsequent entering these microsomal particles.

ACKNOWLEDGEMENT

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ASCORBIC ACID ELECTROCHEMICAL DETERMINATION IN COMMERCIAL PRODUCED JUICES

Viktor JANKOVSKÝ[1], Zuzana KOUDELKOVÁ[1], Lukáš RICHTERA[1, 2], Vojtěch ADAM*[1,2]

[1] *Department of Chemistry and Biochemistry, Faculty of Agronomy, Mendel University in Brno, Zemědělská 1, 613 00 Brno, Czech Republic*

[2] *Central European Institute of Technology, Brno University of Technology, Purkyňova 123, 612 00 Brno, Czech Republic*

vojtech.adam@mendelu.cz

Ascorbic acid is a vitamin important for human health. Detecting the concentration of ascorbic acid using electrochemical methods is a simple and sensitive tool for food, pharmaceutical and clinical analyses. Recommended daily dose for an adult person is around 75 mg. Many people accept ascorbic acid using fruit juices. In this contribution is comparison of amount of ascorbic acid produced in the various commercial juices.

The ascorbic acid, commonly called as „*Vitamin C*“, is frequently used in synthetic form as a dietary supplement in tablet shape and of course is also located in many kinds of fruit in the natural form. Human organism is unable to synthetise vitamin C, so it is necessary to deliver it externally. Vitamin C has a wide range of applications for example as an important antioxidant, involved in synthesis of collagen, supporting growth and in last years a high concentratios (more than 1 g) are used as an alternative treatment of some types of cancer [1]. Because it is necessary to deliver vitamin C externally, many people are interested in its content in food products. Especially in the winter months, people can suffer from vitamin C deficiency caused by lack of consumption of fresh fruit. Even though some producers declare the content of vitamin C, the information doesn't have to be accurate. That's why the goal was to compare declared concentrations with the real ones and also measure the concentration of vitamin C in those, which don't provide the information. Electrochemical detection seemed to be the best way, how to measure it, due to it's reliability and repeatability [2, 4].

The basis of this method is described in Metrohm manual, containing the description of peak determination and used buffer. It was necessary to optimize the method. Our three electrode system was using mercury electrod as a working one, Ag/AgCl (3 M KCl) as reference and platinum electrode as auxiliary. The electrolyte we used was 0.1 M acetate buffer, pH 4.6 [6]. Ascorbic acid undergoes degradation when exposed to air and light so it was needed to measure the samples as soon as it was possible to reach the objective results [5]. All the time the amount of solution containg the sample was the same (5 ml), only the concentration was changed. Every single measurement required new sample preparation. To find out a concentration in juices it was necessary to measure the standart solution of ascorbic acid. Than a calibration curve was made of standart solution and than the concentrations were counted by substituting average peak heights [3].

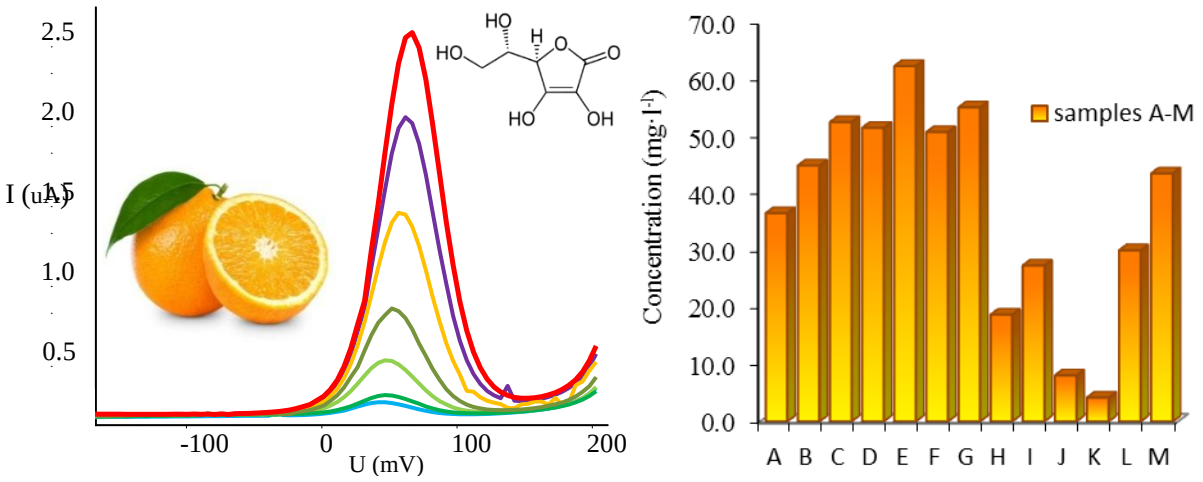

Figure: Voltammograms of ascorbic acid in fresh orange and the comparison of the ascorbic acid concentration in commercial produced juices (samples A – M).

The samples, in which concentrations were declared, didn't reach the required results in any sample. The concentrations in fruit juices were measured easily, measurements were reproducible and were not influenced by any disturbing factor, and this fact was verified by standard addition method. We also found out, that measured amount of ascorbic acid in vegetable juices was influenced to some extent by other substances. The main point of interest were orange juices, due to their availability. For orange juices doesn't apply, that the most expensive ones contain the highest amount of ascorbic acid, because some producers are adding it in synthetic way. In some kinds of fruit juices ascorbic acid was undetectable due to the concentration forthcoming zero.

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DEAE/SDS AND CHITOSAN/SDS HYDROGELS

Sabína JARÁBKOVÁ, Jiří SMILEK, Filip MRAVEC, Miloslav PEKAŘ
Materials Research Centre, Brno University of Technology, Purkyňova 464/118, 612 00 Brno, Czech Republic
**xcjarabkova@fch.vut.cz*

Abstract
This work is focused on ionic interactions between oppositely charged polyelectrolytes and surfactants which can – under proper conditions – lead to formation of physical gels. Surfactants form micellar structures enabling to solubilize hydrophobic substances in the otherwise aqueous environment of the gel. Resulting materials can find use in the formulation of delivery systems, e.g. hydrophobic drugs especially in topical applications. Main aim of this work is to study rheological properties and preparation techniques of materials, that are formed from positively charged polysaccharides (chitosan, cationized dextrane) and negatively charged surfactant (SDS). The presence of hydrophobic nanodomains was tested by solubilization of hydrophobic dyes (e.g. Oil red O, prodan).

INTRODUCTION

Due to electrostatic interaction between polyelectrolytes and oppositely charged surfactant various types of colloids, nanocolloids or even bulk can be formed [1]. Hydrogels are colloidal materials which can be, under proper conditions, prepared by previously described way. Dispersion phase in gels is in liquid state and dispersed phase (colloidal "particles") is in solid state. Hydrogels can be formed at sufficiently high concentration of surfactant. The surfactant micelles can act as crosslinking points and gelled material is formed.
These hydrogels contain hydrophobic domains (formed by the surfactant micellar structures) in nanometre scale and they should be able to solubilize hydrophobic substances within the otherwise hydrophilic gel matrix. Behavior of gels depends on the density of its network [2]. If there are no biocompatibility issues, gels can be used in applications in different fields related to human health. It includes e.g. pharmaceutical, bioengineering, (bio)medicine, drug delivery. These gel materials can be explained as hybrid – hydrophilic-hydrophobic – materials from the point of view of polarity of their constituents [2].

RESULTS AND DISCUSSION

Rheological experiments did not reveal substantial effect of the type of aqueous environment on the viscoelastic properties of the formed gel materials. Figure shows the dependence of both rheological moduli on the deformation frequency. Mechanical properties (toughness) of the gels can be controlled in a broad range by the composition of the gelling system. The elastic (storage) module was well above the viscous (loss) module over the whole tested frequency range for all samples. This clearly demonstrates dominating elastic response, i.e., the gel character, of prepared materials. Further, the moduli are almost independent of the deformation frequency. This is typical for densely crosslinked polymers, rigid gels or dispersions [3]. Composition has significant effect on the rheological properties of the gels; their toughness as measured by the moduli values decreased in the order of the sample numbers: $1 > 4 \approx 2 > 3$. Thus, the high concentration of both polyelectrolyte and surfactant is necessary for high toughness. On the other hand, the surfactant concentration seems to be

more significant for the preparation of weaker gels, as the surfactant nanodomains are supposed to serve as the gel crosslinking nodes.

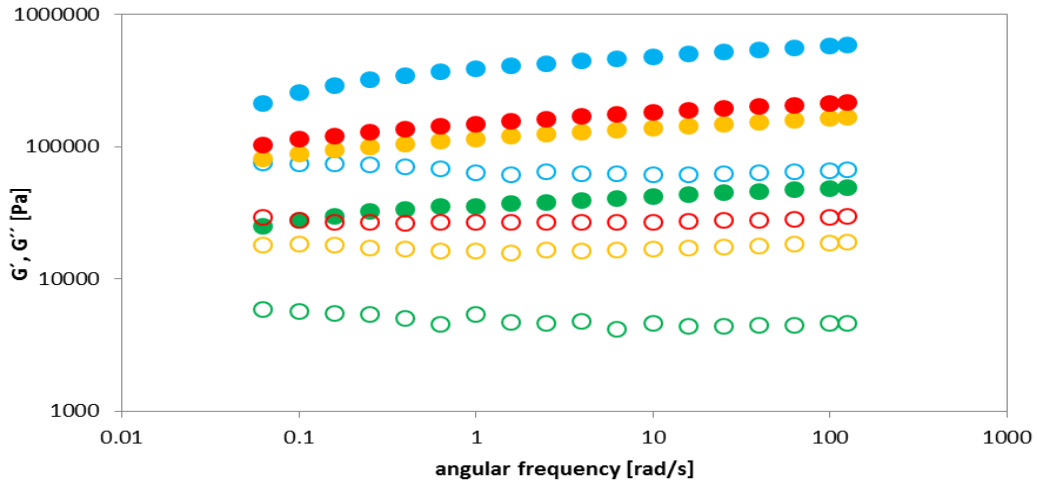

Figure: Storage (filled symbols) and loss (empty symbols) moduli of chitosan gels prepared in 0.15 M NaCl; sample 1 (blue), 2 (orange), 3 (green), and 4 (red).

CONCLUSION

Cationic polyelectrolytes and anionic surfactants can form physically crosslinked hydrogels (under proper conditions). Resulting gel materials contain hydrophobic nanocontainers which can solubilize hydrophobic compounds. Structure and rheological properties depend on the concentration of polyelectrolyte and surfactant. These materials can be used in many applications, especially in medicine for drug delivery systems or topical treatments.

ACKNOWLEDGEMENT

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ANTIBODY TREATMENT OF LUNGS – INHALATION EFFICIENCY

Božena KUBÍČKOVÁ*, Kateřina DOSTÁLOVÁ, Marie STIBOROVÁ, Petr HODEK
Department of biochemistry, Faculty of Science, Charles University in Prague, Hlavova 8, 128 40 Prague 2, Czech Republic
**bojkaku @gmail.com*

Respiratory system of the cystic fibrosis (CF) patients is affected by the defect in the gene coding for protein transporter of chloride ions – CFTR ("Cystic fibrosis transmembrane conductance regulator"). The main complication of this disease is chronic inflammation of CF airways, in particular caused by bacterium *Pseudomonas aeruginosa*. These infections are usually treated with antibiotics, but the bacteria become resistant against them and so that new methods of CF patient therapy are needed. Prophylaxis by vaccination could be one of the possible alternatives. Vaccines for active immunization of CF patients are designed to prevent PA adhesion [1]. The main advantage of this approach is that the bacteria can not develop antibody resistance. Whereas these vaccines are capable of inducing the antibody production, the antibodies mostly do not reach the site of PA infection in sufficient amounts. Passive immunization appears more suitable, when completed antibodies are administered directly to weakened CF patients. One possibility of passive immunization is application of hen yolk IgY antibodies developed against PA virulence factors. The effect of orally administred IgY has already been proven [2]. In our study we dealt with the possibility of CF patients immunization via the *inhalation* of IgY in the *form* of *aerosol.* Inhalation enables the antibody application directly to lungs. The aim of our experiments was to quantify the antibody amount, that is actually inhaled into the respiratory system of experimental animals.

Lectin PAIIL, virulence factor of *Pseudomonas aeruginosa*, was prepared by recombinant expression in *E. coli* (M. Wimmerová, MUNI, Brno). Recombinant PAIIL lectin was subsequently applied for hens immunization and IgY fractions were prepared from collected egg yolks as described elsewhere [3]. To quantify inhaled antibodies, FITC (fluorescein isothiocyanate) labeled IgY were used. Mouse strain ICR CD1 was used as an animal model. At first, known dose of IgY was applied by intratracheal instillation into the trachea of mice. After 10 minutes, bronchoalveolar lavage (BAL) was prepared from the lungs of mice. Based on the fluorescence the portion of IgY that can be recovered from the airways by BAL was determined. Next, the mice were exposed to nebulized IgY using two setups: i) individual animal inhalation and ii) inhalation in a group of three animals. The inhalation was carried out using a PARI BOY nebulizer at a rate 2 l / min for 10 minutes. Bronchoalveolar liquid was prepared from the lungs of the exposed mice and the IgY-FITC content was quantified.

The obtained results showed that that about 32% of the total dose administered by intratracheal instillation can be recovered from the lungs of model animals in BAL. These data has been used to estimate the amount of inhaled IgY. We found that a more efficient arrangement with respect to inhaled IgY content is individual inhalation. A smaller space of the exposure chamber allowed inhalation 0.16% of the total IgY dose. Inhalation in the group resulted in doses more than two folds lower (see Table 1). However, even relatively low amount of IgY was inhaled it should be sufficient to be protective when affinity purified IgYs (on immobilized PAIIL) are administrated. Verification of this idea will be the subject of the following experiments.

Table 1: Content of inhaled IgY-FITC in BAL fluid

# animal / method of inhalation		IgY-FITC antibody content in BAL fluid (□g)	Percentage of total inhaled dose 10 mg (%)
group	**1**	2.2	0.07
	2	1.3	0.04
	3	2.5	0.10
individual	**4**	5.0	0.16
	5	5.4	0.17
	6	4.7	0.15

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TERAHERTZ WAVEMIXING IN A CHI(3) MEDIUM

Dusan LORENC[1,2*], Martin KOYS[1], Eva NOSKOVICOVA[2], Lenka SLUSNA[2], Dusan VELIC[1,2]

1 *International Laser Centre, Ilkovicova 3, 84104 Bratislava, Slovak Republic*

2 *Department of Physical and Theoretical Chemistry, Comenius University, Ilkovicova 6, 84215 Bratislava, Slovak Republic*

We have shown by numerically solving a set of generalized nonlinear Schroedinger equations that four-wave mixing between a strong optical pump, a frequency doubled idler and a weak THz seed can result into a significant amplification and spectral broadening of the THz pulses [1]. The non-phase-matched interaction in a dispersive chi(3) medium, a cyclic olefin polymer, produced a short localized temporal THz spike and a corresponding broadband THz spectral pedestal exceeding 10 THz bandwidth. An optimum recipe for the amplification has been identified with respect to signal and idler duration, chirp, THz seed frequency and signal wavelength.

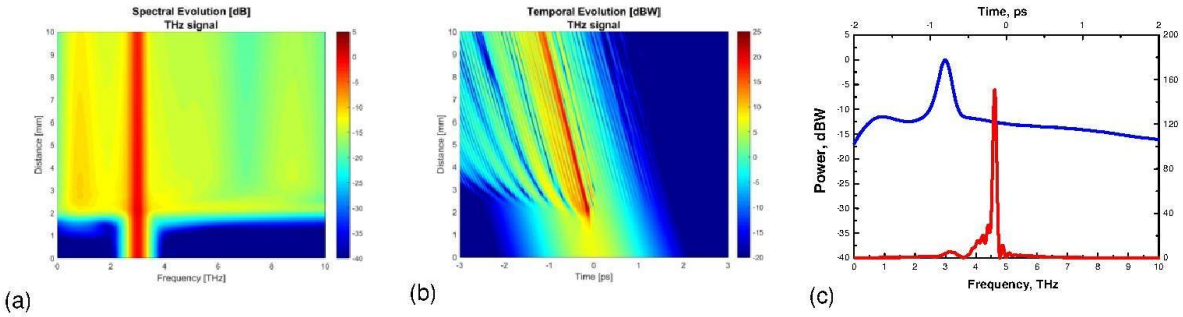

Figure: THz seed pulse propagation dynamics for a 200 fs pump pulse. Spectral (a) and temporal (b) scaling with the propagation distance and the resulting THz pulse (red) and spectrum (blue) (c)

ACKNOWLEDGEMENT

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THE COMPARISON OF THE CELL PROLIFERATION ANALYSIS METHODS

Katsiarina MORGAENKO[1], Larisa BAIAZITOVA[1*], Josef SKOPALÍK[1], Ondřej SVOBODA[1], Vratislav ČMIEL[1], Ivo PROVAZNÍK[1]

1 *Department of Biomedical Engineering, Faculty of Electrical Engineering and Communication, Brno University of Technology, Technická 3082/12, 616 00 Brno, Czech Republic*
*xbaiaz00@stud.feec.vutbr.cz

MTT assay is widely used assay for testing of drug or radiation effects on cell viability and growth. Many laboratories worldwide have adopted a microculture assay (cells in 96-well chambers), based on metabolic reduction of 3-(4.5-dimethylthiazol-2-yl)-2.5-diphenyltetrazolium bromide (MTT); living cells display this reduction and their final optical density is going up [1], quantification of the optical density increase is handled by elisa reader. However, there is one commonly desinterpreted conclusion in some published articles, which assert, that MTT density is proportional to cell numbers. In our experiments, we have found out that this conclusion is valid only in special cases and starting cell density is very important parameter. Details are described in following blocks.

METHODS:

Step 1: Rat fibroblast 3T3 were cultured in DMEM medium with supplement of FBS on plastic chamber. After trypsinization, the specific amount of the cells was seeded to 1.02 cm^2 chamber (in total 24 chambers). The variants of specific amount of the cells is summarized in the Table.

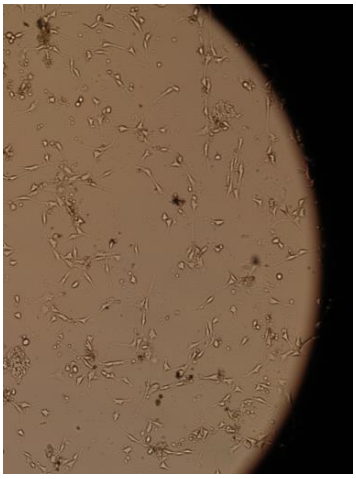
Step 2: In four different time points (day 0, 3, 6 and 9), 3-(4.5-dimethylthiazol-2-yl)-2.5-diphenyltetrazolium bromide (MTT; concentration 0.2 – 0.5 mg/ml) was added to different 4 chambers and analyzed by elisa reader (optical densitometry) 4 hours after MTT application. In the same day, another 4 chambers were not treated by MTT and analyzed by automatic cell counting robotic camera (prototype prepared in our lab). Robot consists of microscope optical part and PC part. The first part is optimized for cell focusing and photographing of large wide field (5000×5000 micrometers). The second part is PC with Matlab utility, which is prepared for exact cell number enumeration and recomputing to cells/cm^2 value.

RESULT:

Typical distribution of fibroblasts in the culture chamber is visible in the Figure (photographed by robotic camera). The results show evidence that high confluent cell culture have cells which have average lower metabolic activity per one cell than non-confluent culture. The final graphs of correlation is summarized in the Figure-B

Table: Cell sample variants

Starting cell density (cells / cm^2)	Time 1	Time 2	Time 3	Time 4
500	Day 0	Day 3	Day 6	Day 9
750	Day 0	Day 3	Day 6	(Overgrowth)

A 

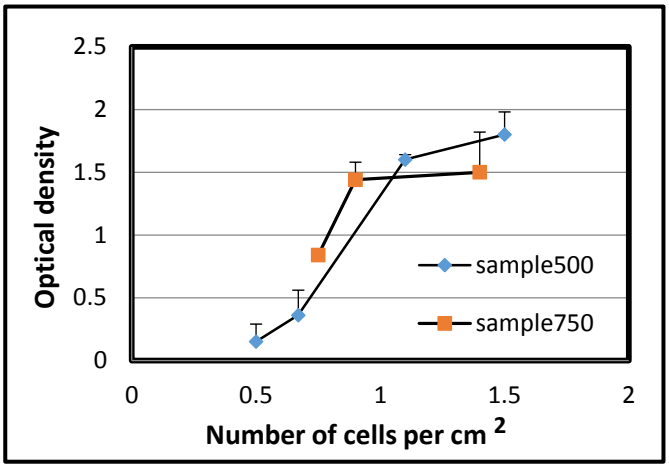
B 

Figure: A – The fibroblast on bottom of the chamber, B – correlative analysis of cell number derived by robotic camera and MTT optical density. The points represent time 1, 2, 3, 4 via Table.

The result brings precise information about correlation of the "MTT assay" with "cell enumeration assay". The result is in agreement with our previous study, where cells seeded with lower density have larger cell bodies and significantly larger area of mitochondria [2]. The results are important for design and interpretation of future complex toxicological in-vitro tests and cell monitoring.

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ENHANCED GENOTOXICITY OF PLANT ALKALOIDS ARISTOLOCHIC ACID I AND II AFTER THEIR COMBINED APPLICATION TO RATS

Marie STIBOROVA[1*], Frantisek BARTA[1], Petr HODEK[1], Jaroslav MRÁZ[2], Šárka DUŠKOVÁ[2], Eva FREI[1], Heinz H. SCHMEISER[3], Volker M. ARLT[4]

[1] *Department of Biochemistry, Faculty of Science, Charles University, Albertov 2030, 128 40 Prague 2*

[2] *Centre of Occupational Health, The National Institute of Public Health, Srobarova 48, 100 42, Prague 10*

[3] *Division of Radiopharmaceutical Chemistry, German Cancer Research Center (DKFZ), Im Neuenheimer Feld 280, 69120 Heidelberg, Germany*

[4] *Analytical and Environmental Sciences Division, MRC-PHE Centre for Environment & Health, King's College London, Franklin-Wilkins Building, 150 Stamford Street, London SE1 9NH, UK*

**stiborov@natur.cuni.cz*

Abstract. A plant extract aristolochic acid (AA) is a mixture of AAI and AAII that causes Aristolochic acid nephropathy, Balkan endemic nephropathy, and urothelial malignancies. The impact of exposure of both the AAI or AAII alone and the combination of both compounds on genotoxicity of AA in rats *in vivo* was investigated. The results demonstrate additive effects of treatment of rats with AAI combined with AAII on AA-genotoxicity.

INTRODUCTION

Aristolochic acid (AA), the natural extract of plants of the Aristolochiaceae family, is a mixture of structurally related nitrophenanthrene carboxylic acids, with two major components aristolochic acid I (AAI) and aristolochic acid II (AAII). AA has been shown to be human carcinogen and the cause of so-called Chinese herbs nephropathy (CHN), now termed Aristolochic acid nephropathy (AAN) and Balkan endemic nephropathy (BEN) [1,2]. Exposure to AA was demonstrated by identification of specific AA-DNA adducts in urothelial tissue of AAN and BEN patients [1,2]. The most abundant DNA adduct detected in patients is 7-(deoxyadenosin-N^6-yl)-aristolactam I (dA-AAI) which causes specific AT→TA transversions. Such AT→TA mutations have been observed in the *TP53* tumor suppressor gene in tumors from AAN and BEN patients [1,2]. AA exposure was also discovered to contribute to the high incidence of upper urinary tract urothelial carcinoma (UUC) in Taiwan population [3]; again, the *TP53* mutational signature in patients with UUC was predominant among otherwise rare AT→TA transversions. But, information is still lacking how drug-drug interactions between AA components, AAI and AAII, influence the AA-induced BEN/UUC development. Hence, the aim of this study was to evaluate the impact of exposure of AAI alone, AAII alone and the combination of both compounds (a ratio of 1:1) on AA-genotoxicity (the AA-DNA adduct formation) in rats *in vivo*.

MATERIAL AND METHODS

DNA adducts were analyzed by ^{32}P-postlabeling as shown previously [2].

RESULTS AND DISCUSSION

The AA-DNA adducts were formed in liver, kidney and lung of rats treated with AAI, AAII and both compounds in combination (a ratio of 1:1). Up to three AA-derived DNA adducts were formed in these rat organs; dA-AAI, 7-(deoxyguanosin-N^2-yl)-aristolactam I (dG-AAI),

7-(deoxyadenosin-N^6-yl)-aristolactam II (dA-AAII) and 7-(deoxyguanosin-N^2-yl)-aristolactam II (dG-AAII) (Figure 1).

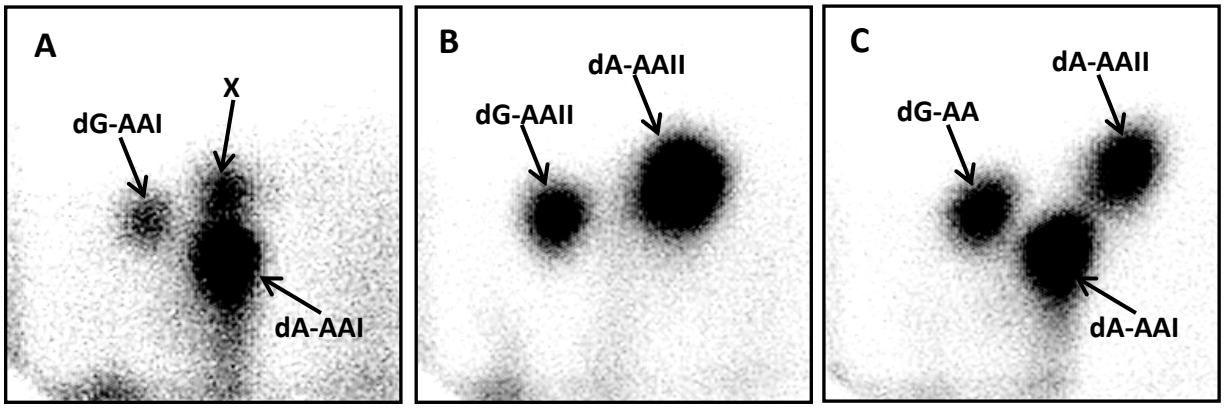

Figure 1: AA-derived DNA adducts formed in livers of rats treated with AAI (**A**), AAII (**B**), and AAI combined with AAII (**C**). DNA adducts were determined by ^{32}P-postlabeling [2].

The same levels of AAI- and AAII-derived DNA adducts were found in liver of rats treated with AAI or AAII, but up to 2.8- and 2.1-fold higher levels of AAII- than AAI-derived DNA adducts were formed in kidney and lung of rats treated with either compound, respectively. Compared to rats treated with AAI or AAII alone, the sum of total levels of AA-DNA adducts were 2.4-, 1.7- and 2.7-fold higher in liver, kidney and lung, respectively, of rats treated with both AA components in combination than the levels of AAI- and AAII-DNA adducts formed after exposure to these compounds individually. The results demonstrate the additive effects of exposure of rats to AAI combined with AAII on AA-genotoxicity.

ACKNOWLEDGEMENT

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TiN MICROELECTRODE CHAMBER COATING FOR IMPROVED CARDIOMYCYTE CELL ADHESION AND ELECTROPHYSIOLOGICAL CHARACTERIZATION

Ondrej SVOBODA[1,*], Josef SKOPALIK[2], Larisa BAIAZITOVA[1], Zdenka FOHLEROVA[3,4], Jaromir HUBALEK[3,4,5], Ivo PROVAZNIK[1], Eva GABRIELOVA[6]

1 *Department of Biomedical Engineering, Faculty of Electrical Engineering and Communication, Brno University of Technology, Brno, Czech Republic*
2 *Department of Human Pharmacology and Toxicology, University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic*
3 *Central European Institute of Technology, Brno University of Technology, Brno, Czech Republic*
4 *Department of Microelectronics, Faculty of Electrical Engineering and Communication, Brno University of Technology, Brno, Czech Republic*
5 *SIX Centre, Department of Microelectronics, Faculty of Electrical Engineering and Communication, Brno University of Technology, Brno. Czech Republic*
6 *Department of Medical Chemistry and Biochemistry, Faculty of Medicine and Dentistry, Palacky University, Olomouc, Czech Republic*
**xsvobo32@stud.feec.vutbr.cz*

Neonatal cardiomyocytes are a widely used model for in-vitro morphological, biochemical, toxicological, electrophysiological characteristics and interactions of heart cells [1,2]. The recording of neonatal myocyte actions and analysis of the signals from single cells, small aggregates and complete cell clusters and cultures in real time is one of the most current methods in modern electrophysiology. This study is focused on the impact of using different substrates in culture chambers on the surviving and functional electrophysiological activity of the myocytes five days from isolation. The microelectrode arrays (MEAs) systems, one of the most modern tools, are based on extracellular recordings and have many advantages over traditional methods. However, the MEA samples preparation can negatively affect the results from mathematical modelling of electrophysiology and future biomedical applications. The coating of the microelectrode array has a significant role in cell signaling and the development of cardiomyocyte electrophysiology during the first few days after seeding.

The TiN 120-electrode MEA chambers (12×12 electrode layout, 4 reference and 4 ground electrodes Multi-Channel Systems) were coated O/N in four ways (see Table). After that the cardiomyotes isolated from two-days old neonatal rats by trypsin digestion (0.2% w/v) were seeded at density 4×10^5 cells/cm^2 and cultured in DMEM at 5% CO_2 and 37°C for 120 hours. 120 channels USB 2.0 MEA2100-System (Multi-Channel Systems) was used for electrical recording, with 25 kHz sampling frequency and ±10 mV input range. The different biphasic electrical pulses (250, 500, 1000 and 2000 mV amplitude, 16.67, 100, 200 and 333.33 Hz frequency) from internal stimulus generator were used to evoke electrical response. Responses were analyzed in point of shape, frequency and amplitude.

Table : The MEA chamber neonatal cardiomyocytes adhesion

Surface treatment	Adhesion (mean±SD)	Evoked esponse
None	1.0±0.1	No
Collagen II	4.9±0.5	No
PDL	18.0±1.5	No
PDL+fibronectin	31.5±1.2	Yes
PDL+laminin	38.7±5.8	Yes

Results showing high viability of adhered cells (> 95%, Calcein AM method) with no detectable spontaneous activity in any of coating method. However, on PDL+fibronectin and PDL+laminin coated MEA chambers, the evoked electrical response from neighboring evoked cell electrical responses with typical shape were detected at the amplitudes 198±18 mV and 179±12 mV respectively (20 cells, 20±5 ms between pulses) with ho significant cell desensitization in time stimulation. Notwithstanding these methods also display over 30% cell adhesion, conductivity of depolarization pulses over cell culture was not been identified.

Our results reveal the importance of carefully selecting the substrate coating for cardiomyocyte adhesion and electrophysiological characterization. In future, wewish to investigate another modern coating based on naturally occurring complete extracellular matrix.

ACKNOWLEDGEMENT

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THERMODYNAMIC AND KINETIC STUDY OF 14-3-3 ζ DIMERIZATION

Zuzana TROŠANOVÁ[1], Petr LOUŠA[1], Tomáš BROM[1], Veronika WEISOVÁ[1], Gabriel ŽOLDÁK[2], Jozef HRITZ[1*]

1 *Central European Institute of Technology, Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic*

2 *Technical University Munich, James-Franck-Straße 1, 85748 Garching bei München, Germany*

* hritz@ceitec.muni.cz

14-3-3 proteins are regulatory proteins involved in many signaling pathways. They play a key role in nervous system and neurodegeneration.[1-3] The 14-3-3 family of proteins consists of seven isoforms in mammals and interact with large number of binding partners[4] (Aitken, 2006). All isoforms recognize protein with identification of two optimal phosphoserine/threonine-containing motifs, RSXpSXP and RXXXpSXP, where pS means phosphoserine/threonine. The three dimensional structure showed 14-3-3 dimeric structure. Each monomer consits of 9 α-helices in antiparallel arrangement. Dimer interface is stabilized by multiple conserved hydrophobic interactions (e.g. L12), polar contacts and by several isoform-specific salt bridges (e.g. K78).[5,6] However, dimer dissociation constants as well as fundamental kinetic rate constants are unknown.

In order to study kinetics of 14-3-3zeta dimer, we prepared a new construct with a single accessible cysteine at the N terminus of protein. This construct was used in self-quenching assay (SQ) and in microscale thermophoresis (MST) methodology. The SQ design is based on the close proximity of two TMR molecules in the dimer results in quenching their fluorescence. In the monomeric state, TMR molecules are isolated and thus their fluorescence is higher. This approach was used for determination of kinetic parameters (biomolecular association rate constant kon and dissociation rate constant koff). The thermodynamic parameter (dissociation constant, Kd) was determined in nanomolar range using MST.

ACKNOWLEDGEMENT

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OXIDATION OF AN ANTICANCER DRUG VANDETANIB BY RAT CYTOCHROMES P450 AND FLAVIN MONOOXYGENASES *IN VITRO*

Radek INDRA[1], Katarina VAVROVÁ[1], Vlastimil HROMEK[1], Petr POMPACH[1], Zbyněk HEGER[2], Vojtěch ADAM[2], Marie STIBOROVÁ[1*]

[1] *Department of Biochemistry, Faculty of Science, Charles University, Albertov 2030, 128 40 Prague 2, Czech Republic*

[2] *Department of Chemistry and Biochemistry, Laboratory of Metallomics and Nanotechnology, Mendel University in Brno, Zemedelska 1, 61300 Brno, Czech Republic*

**stiborov@natur.cuni.cz*

Abstract. The study investigates the metabolism of vandetanib [one of the inhibitors of tyrosine kinases (TKIs)] *in vitro*, by biotransformation enzymes of a rat animal model. Using rat hepatic microsomes enriched with individual cytochrome P450 (CYP) enzymes by their induction, the inhibitors of these enzymes and flavin monooxygenases (FMOs) as well as rat recombinant CYPs, the oxidation of vandetanib was determined. The metabolites of vandetanib were identified by mass spectroscopy to be *N*-desmethylvandetanib and vandetanib-*N*-oxide. The CYPs of the 3A and/or 2C subfamilies form mainly *N*-desmethylvandetanib, whereas FMOs are responsible for generation of vandetanib-*N*-oxide.

INTRODUCTION

The studies carried out in last decades demonstrate that the drugs used for cancer chemotherapy have a narrow therapeutic index, and often the produced responses are only palliative as well as unpredictable. Such approaches, although directed toward certain biomacromolecules, do not discriminate between rapidly dividing tumor *vs.* non-malignant cells [1]. In contrast, targeted therapy that has been introduced in recent years is directed against cancer-specific targets and signaling pathways, and thus provides more limited nonspecific mechanisms [2]. The most promising targets are receptor tyrosine kinases (TKs), the enzymes that selectively phosphorylate the hydroxyl moieties of tyrosine residues on signal transduction molecules with a phosphate moiety from adenosine triphosphate [3]. Although TKIs are considered as well-tolerated, various side effects are common [4]. Overall, in cancer chemotherapy, serious clinical consequences may occur from small alterations in drug metabolism affecting drug pharmacokinetics [5]. Nevertheless, there is only little insight in the metabolism of TKIs at this point, which is surprising since they are used on a daily basis in hundred thousands of patients. Thus, the aim of this study was to investigate the metabolism of one of the TKIs, vandetanib, the compound acting as an inhibitor of TKs of numerous cell receptors and using for treatment of certain tumors of the thyroid gland.

MATERIAL AND METHODS

HPLC was utilized for separation of vandetanib metabolites and mass spectroscopy for their structural characterization.

RESULTS AND DISCUSSION

Based on the preliminary pharmacokinetic studies, vandetanib was suggested to be oxidized in human by cytochrome P450 3A4 (CYP3A4) to *N*-desmethylvandetanib, while another metabolite, vandetanib-*N*-oxide, was considered to be formed by flavin monooxygenases (FMOs) [6]. These preliminary results need, however, to be confirmed. Here, we used the rat enzyme systems to investigate the vandetanib oxidation in detail.

Using rat hepatic microsomes enriched with individual CYP enzymes by their induction, inhibitors of these enzymes and FMOs, as well as rat recombinant CYPs expressed in Supersomes™, the metabolism of vandetanib was investigated. Up to two metabolites formed by these systems were separated by HPLC and characterized by mass spectroscopy. Of these metabolites, vandetanib-*N*-oxide was mainly generated by microsomes of control (uninduced) rats, followed by those induced with PCN (an inducer of CYP3A) and ethanol (increasing levels of CYP2E1) and those of benzo[a]pyrene inducing CYP1A1/2. *N*-desmethylvandetanib was predominantly formed by enzymes of microsones induced by PCN. Whereas the formation of vandetanib-*N*-oxide is strongly decreased by inhibitors of FMOs, ANTU and methimazol, the generation of *N*-desmethylvandetanib was attenuated mainly by inhibitors of CYPs of the 3A and 2C subfamilies (ketoconazol and sulfaphenazole). These results suggest that FMOs are mainly responsible for the formation of vandetanib-*N*-oxide, while CYP3A and 2C for that of *N*-desmethylvandetanib. Of all tested rat CYP enzymes, only CYP2C11, 3A1/2 and 2D1, mainly in the presence of cytochrome b_5, were able to oxidize vandetanib to *N*-desmethylvandetanib. No vandetanib-*N*-oxide was generated by tested rat CYPs. The results found in this study confirmed the preliminary finding suggesting that vandetanib is oxidized to *N*-desmethylvandetanib and vandetanib-*N*-oxide in reactions catalyzed by CYP and FMO enzymes, respectively. Further study utilizing human enzymes is underway in our laboratory.

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