Programme and Book of Abstracts

SMCBS'2005 Workshop

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Introduction

The present booklet contains programme and abstracts of invited tutorial lectures, as well as contributed keynote lectures, short oral communications, and posters of the 2nd International Workshop on 'Surface Modification for Chemical and Biochemical Sensing', SMCBS'2005. We are pleased and feel honoured to host over a dozen of distinguished scientists who have kindly accepted our invitation to present tutorial lectures.

This Workshop is organized as a part of the activity of the Centre of Excellence 'Surface Phenomena and Reactions' (SURPHARE) established within the 6th Frame Programme of the European Commission at the Institute of Physical Chemistry of the Polish Academy of Sciences in Warsaw, Poland.

Interdisciplinary between chemistry, biochemistry, molecular biology, physics, materials science, microelectronics, and engineering has created important new ideas in several research fields including sensing and biosensing. For sensing, surfaces of solid substrates of chemical or biochemical sensors are modified for selective or, in some cases, even specific sensing.

The use of sensor-based analytical procedures, originally focused on chemical and biochemical tests, is gaining increasing interest, among others, in environmental toxicity testing, for ecosystem monitoring, clinical diagnosis and therapy, as well as testing of crops and foods of animal origin.

The recent increase of interest in sensor-based analytical techniques is manifested by the increase of the number of both original research papers published and patents registered. Toward this interest, a series of our Workshops is organized. Being encouraged by success of *The 1st International Workshop on Surface Modification for Chemical and Biochemical Sensing, SMCBS'2005*, organizers of the present Workshop are hoping that also *The 2nd Workshop* will successfully offer a platform for researchers to meet for exchanging and generating ideas that will stimulate new, and most expectantly, collaborative research.

Prevailingly, the Workshop is focused on the art of both chemical and non-chemical decorating of surfaces and recognition activity of the resulting sensors toward target analytes.

Main topics of the Workshop will cover various aspects of surface chemistry related to sensing and biosensing in solutions or gases but are not limited to:

● Chemical surface reactions

● Self-assembled monolayers (SAMs)

● Langmuir and Langmuir-Blodgett (LB) films

● Preparation and properties of supported thin films

● Chemically modified electrodes and polymer modified electrodes

● Novel techniques and instrumentation for examining surfaces

● Signal processing, detection techniques, system miniaturization and nanotechnology use.

All participants are accommodated at one Workshop site. Therefore, the number of participants is limited by capacity of this site. Accommodation of all participants at one site facilitates mutual contacts, both formal and informal, enabling discussions to be continued far beyond the program.

Particularly, young researchers, i.e. graduate students, postdoctoral fellows and research assistants are welcome to contribute their enthusiasm and ideas to the field of chemical and biochemical sensing. All presentations, and particularly those of young researchers, are expected to be widely discussed within the audience while constructive inputs of senior scientists are likely very much.

A half-day bus excursion to The Zamoyski Museum in Kozłówka will bring participants closer together for better personal acquaintance and ad hoc discussions in small groups.

The Organising Committee thanks all those who contributed to the workshop. Our gratitude is particularly due to the authors of the contributions, to the chairpersons and members of the International Advisory Board. Moreover, the European Commission is gratefully acknowledged through the SURPHARE Centre of Excellence for financial support of the present scientific event.

On behalf of the Organising Committee, we welcome all Workshop participants and readers of the present booklet.

Włodzimierz Kutner

Chairman

Organising and Programme Committee

Organisers

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- Professor Aleksander Jabłoński
  Director of the Institute of Physical Chemistry of the Polish Academy of Sciences Warsaw, Poland
- Professor Janusz Flis
  Coordinator of the SURPHARE Centre of Excellence at the Institute of Physical Chemistry of the Polish Academy of Sciences, Warsaw, Poland
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Institutions

Institute of Physical Chemistry

The Institute of Physical Chemistry of the Polish Academy of Sciences in Warsaw, Poland was founded on March 19, 1955. It was the first research institute for chemical sciences in Poland. The present scientific profile of the Institute is strongly linked to contemporary directions in physical chemistry and chemical physics. The research work covers a wide range of physicochemical disciplines. It is carried out within 10 research departments. The main topics presently investigated at the Institute are:

- Supramolecular chemistry - structure and properties; X-ray structure determinations, separation techniques, sorption/desorption processes, surface aggregation (Prof. J. Lipkowski, Dr. K. Suwińska, Dr. R. Luboradzki, Dr. A. Bielejewska, Dr. M. Asztemborska), synthesis of supramolecular compounds (Assoc. Prof. M. Pietraszkiewicz).
- Thermodynamics, thermochemistry and biocalorimetry (Prof. W. Zielenkiewicz); thermodynamics of liquids (Assoc. Prof. J. Gregorowicz), development of transitiometry as a tool for study of EOS and phase transitions (Assoc. Prof. S. Randzio).
- Physical chemistry of solid state and surface, examined by various electron spectroscopy techniques (Prof. A. Jabłoński) and scanning tunnelling microscopy techniques (Dr. R. Nowakowski).
- Photophysics and photochemistry of organic molecules, involving spectrally and time resolved laser techniques (Prof. J. Waluk, Prof. Z. R. Grabowski, Prof. A. Grabowska, Prof. K. Rotkiewicz, Assoc. Prof. J. Herbich, Assoc. Prof. A. Kapturkiewicz, Assoc. Prof. J. Sepioł, Assoc. Prof. J. Dobkowski, Assoc. Prof. R. Kołos).
- Studies of interaction of gas phase (mainly hydrogen) with surface of metals and alloys, especially during metal-semiconductor transition that may occur in bulk material (Prof. R. Duś).
- Preparation and characterization of highly dispersed mono- and bimetallic catalyst systems, aided by X-ray diffraction analysis (Prof. Z. Karpiński, Prof. J. Pielaszek, Assoc. Prof. A. Borodziński, Assoc. Prof. J. Zieliński); structure of nanocrystalline systems: modelling and experiment (Assoc. Prof. J. Pielaszek, Dr. Z. Kaszkur, Dr. B. Mierzwa); catalysis for environmental problems (Prof. Z. Karpiński, Prof. J. Pielaszek).
- Statistical mechanics in application to soft matter (Prof. R. Hołyst, Prof. J. Stecki, Prof. A. Ciach, Dr. A. Poniewierski).
- Electrochemistry, electrode processes, electrochemical sensing and sensors (Prof. J. Taraszewska, Assoc. Prof. P. Żółtowski, Prof. W. Kutner, Prof. M. Opałło); electrochemical oxidation of gaseous fuels (Prof. L. Rostwo-Suski);
- Theory of chemical kinetics (including periodical reactions), aided by computer simulations (Assoc. Prof. A. Kawczyński, Prof. J. Górecki).
- Quantum theory of the solid and the molecule: theory of density functional, chemical reactivity indices (Prof. A. Holas, Dr. M. Cinal, Dr. R. Balawender); theory of magnetic thin films and 2D crystalline systems (Prof. S. Olszewski, Dr. M. Cinal, Dr. T. Roliński); hydrocarbons with unusual spatial structure; molecular modelling and NMR spectroscopy studies of cyclodextrins, fullerenes and carbon nanotubes (Prof. H. Dodziuk).
- Corrosion, modification of metal surface for corrosion protection; hydrogen in metals (Prof. J. Flis, Prof. E. Łunarska, Prof. T. Zakroczymski, Prof. M. Janik-Czachor).
- Kinetics and mechanism of chain reactions in homogeneous/heterogeneous systems in relation to environmental transformations of sulphur dioxide: physico-chemical fundamentals of desulphurisation processes; atmosphere chemistry including effects of organic and inorganic scavengers of suffix radicals; interactions between these radicals and bio-organic compounds (Prof. W. Pasiuk-Bronikowska, Dr. T. Bronikowski, Dr. K. J. Rudziński, Dr. J. Ziajka).

- Physical chemistry of metal-hydrogen systems: synthesis of novel materials under high-pressure conditions and investigations of their properties; determination of EOS and search for pressure-induced phase transitions in hydrides (Prof. B. Baranowski, Assoc. Prof. S. M. Filipek, Assoc. Prof. M. Tkacz).

Technological section of the Institute, the CHEMIPAN Laboratories (headed by M.Sc. M. Cieślak), develops, manufactures and commercialises fine and specialty chemicals as well as products for agriculture and pharmacy, including pheromones ("biological traps" for protection of forests and crops), and new drugs.

Annually, the Institute publishes ca. 300 original chemical research papers in peer-reviewed quality journals.

In addition to research, the Institute fulfils its educational mission, both at the under-graduate and graduate level. A Ph.D. Program was launched in 1965. It provides training in both physical and theoretical chemistry. Since 1965, 300 Ph.D. degrees have been awarded. Now, main research topics of the Ph.D. Program include:

- Nanotechnology and new materials
- Photochemistry, photophysics and molecular spectroscopy
- Physical chemistry of surfaces and interfaces, of solids (reactions under high pressure, structural studies), of soft matter, and of supramolecular complexes
- Chemical kinetics and catalysis, thermodynamics
- Quantum chemistry, molecular dynamics and modelling
- Electrochemistry of electrode processes, chemical and biochemical sensors, and protection against corrosion
- Astrochemistry

In 1993, the Institute co-founded the College of Science for under-graduate education. Now, this College operates as the Department of Mathematics and Natural Sciences of the Cardinal Stefan Wyszyński University in Warsaw. Over 30 % of the Institute research personnel are involved in educational activity of this Department. Moreover, the Institute collaborates with Chemistry Departments of the Warsaw University and Warsaw University of Technology in their M.Sc. programs.

SURPHARE Centre of Excellence

General Information

The 'Surface Phenomena and Reactions' (SURPHARE) Centre of Excellence of the Institute of Physical Chemistry was established for networking co-operation of different scientific institutions and strengthening links between centres working in the field of surface science. The Centre's goal is to increase competence of its research personnel in order to become one of the leading institutions in the field attracting both top scientists and young researches.

Surface science and surface engineering have rapidly been developing since early sixties of the last century. This fast development was stimulated by progress in vacuum technology. Surface science and surface engineering include studies of formation of surfaces with defined chemical composition, structure and physical properties as well as studies of phenomena and chemical reactions at surfaces. The research results allow for selecting technology most suitable for preparing materials with well-defined surface properties.

Surface phenomena occurring at the boundaries of solids, liquids and gases play an important role in industrial processes and their recognition can influence engineering projects. The processes important in industry and technology include chemical catalysis, corrosion protection, power industry (fuel and solar cells), processing of plastic materials, modern electronics of integrated circuits, industry of machine design, and systems of selective detection (micro-sensor membranes using modern selective receptor systems).

Solving mechanisms of the physicochemical processes, which run under known thermodynamic conditions at the phase boundaries, allows to control formation or modification of the surface with well defined properties. In order to determine these properties, it is necessary to perform studies using wide range of experimental and theoretical methods that permit to characterise the surface as well as to monitor and describe the processes occurring on them. The experimental techniques for surface studies available in the Centre include Auger electron spectroscopy (AES), photoelectron spectroscopy (UPS and XPS, ESCA), infrared spectroscopy (IR), isotherm desorption spectroscopy (IDS) and thermally desorption spectroscopy (TDS), atomic force microscopy (AFM), scanning tunnelling microscopy (STM), low energy electron diffraction (LEED), X-ray diffraction (XRD), and Langmuir-Blodgett (LB) trough facility. The Centre is engaged in the following main research activity.

- Heterogeneous catalytic reactions - involve preparation of new compounds of the required physico-chemical properties and controlling elementary steps of surface processes that occur during reaction. Efficiency and selectivity of the catalyst are determined by formation of expected forms of reagents on the catalyst surface. The following aspects of these studies are emphasized: quantitative description of chemical composition of the surface, definition of the surface structure in atomic and nanometre scale including vacancies and defects as well as adsorbed molecules, construction of novel catalysts, in which the active particle is introduced into the selected place in the catalyst carrier.

- Metal hydrides - formation of hydrides by using a high-pressure technology and electrochemical methods; adsorption of hydrogen and its ingress into metals.
- Electron spectroscopy - surface analysis by AES, XPS, and elastic scattering; theory of elastic backscattering of electrons on surfaces.
- Evaporated metal layers - chemical reactivity.
- Corrosion - surface modification for enhanced protection against corrosion.
- Thin films for chemical and biochemical sensing - preparation and property studies of both the molecular-layer-thick and polymer surface films on solid electrodes for simultaneous electrochemical and piezoelectric microgravimetry sensing of target analytes.

Scientists of the Centre collaborate with their partners in industrial enterprises in evaluating, designing the apparatus and elaborating the technological procedures. Establishing of the SURPHARE Centre of Excellence results in bringing together scientists of the Institute engaged in surface science and facilitates collaboration with industry.

The aims of the Centre are, as follows.

- Development of scientific co-operation with other surface science laboratories in Europe
- Intensification of personal contacts
- Exchange of information related to recent advances in surface science
- Increase of the education level and research experience of young scientists.

These objectives are realised by organising international conferences and workshops as well as by exchanging research personnel. Scientists and PhD students of the Institute are sent to other research institutions for training and for gaining experience in the advanced research techniques. Also, researchers from other institutions are hosted in the Centre for training and for a join study in surface science.

The conferences and workshops focus on surface phenomena in corrosion, on characterisation and modification of surfaces for enhancement of their catalytic activity and the ability of selective sensing of analytes of interest. A work is also conducted to strengthen the co-operation on standardisation and quantification of the surface science techniques.

Management and administration

The Centre is managed by Prof. Janusz Flis, a former head of the Electrochemistry and Corrosion Department of the Institute and the Centre Coordinator. The International Advisory Board, Coordination Board, and Task Group Leaders support his work. The Institute, whose part the Centre remains, also supervises the Centre.

Management structure of the Centre

(i) The International Advisory Board of the SURPHARE Centre consists of five members, i.e. the Director of the Institute of Physical Chemistry, Prof. Aleksander Jabłoński, and four distinguished scientists from other European research centres.

- Prof. Dr. B. Gruzza, Surfaces and Interfaces Group, Physics Department, Blaise Pascal University, Clermont-Ferrand, France
- Prof. Dr. Waldfried Plieth, Department of Mathematics and Nature Sciences of the Institute of Physical Chemistry and Electrochemistry, Dresden Technical University, Dresden, Germany
- Prof. Dr. Sven Tougaard, Physics Department, University of Southern Denmark, Odense, Denmark
- Prof. Dr. K. Wandelt, Institute of Physical and Theoretical Chemistry, Bonn University, Bonn, Germany.

(ii) The Coordination Board comprises all Task Group Leaders:

- Prof. Janusz Flis (WP1 and WP7)
- Prof. Stanisław Filipek and Prof. Maria Janik-Czachor (WP2)
- Dr. Anna Maciołek (WP3)
- Prof. Włodzimierz Kutner (WP4)
- Prof. Aleksander Jabłoński (WP5)
- Dr. Iwona Flis-Kabulska (WP6)

and also other researcher workers of the Institute.

Objectives

The importance of solid surfaces is growing continuously in different areas of technology, such as corrosion science and engineering, advanced materials for new technology processes, and engineer structures including design of novel catalysts, modern microelectronics, and new polymers. Research in this area requires multidisciplinary approach to problem solving and highly sophisticated instrumentation. Because of relatively high cost of this instrumentation for analysis and modification of surfaces, the surface research should be conducted jointly by the organisations hiring highly qualified personnel and equipped with that instrumentation. Accordingly, the main objective of the Centre activity is to promote scientific collaboration between laboratories, which are well equipped with adequate instrumentation.

Innovative character

The innovation offered by the Centre consists mainly in intensive dissemination of knowledge in the field of surface science, and in standardisation of measurement techniques. Emphasised is surface analysis, surface modification, characterisation of surface processes, and presentation of advanced research techniques. These goals are realised by organising scientific symposia and workshops. The work is carried out on standardisation and quantification of surface science techniques. Large effort is put on training of young scientists in using advanced techniques for surface characterisation and modification.

Work plan

Activities of the SURPHARE Centre are grouped in seven Work Packages, i.e. WP1 through WP7.

Four packages, WP1 through WP4, involve scientific meetings. In total, five conferences and workshops are being organised during three-year period of the Centre activity. The aim of these meetings is to provide information on advances in surface science, to share experience in the modern experimental techniques, and to launch join research proposals for the nearest future. Internationally renowned scientists from European and other countries are invited to the conferences and workshops to teach and pass their knowledge to students and young researchers. Particularly, young scientists are strongly encouraged to participate in the meetings.

Work Package WP5 is foreseen to strengthen co-operation on standardisation and quantification of surface science techniques, whereas Work Package WP6 focuses on training of Centre's scientists and Ph.D. students by co-operation with internationally renowned laboratories. Work Package WP7 involves the management of the Centre.

- WP1: Conference 'Advances in Corrosion Science and Application' main themes: (i) Surface modification for corrosion protection; (ii) Modern surface analytical techniques in corrosion science, Zakopane, September 2003.
  Person responsible in the Centre: Prof. Janusz Flis.
- WP2: Conference 'New Methods of Modification of Reactivity of Amorphous and Nanocrystalline Materials' Warsaw, Summer 2003.
  Persons responsible in the Centre: Prof Stanisław M. Filipek and Prof. Maria Janik-Czachor.
- WP3: 'Workshop on Morphology of Surfaces and Interfaces in Soft Matter. Fundamentals and Applications', Jadwisin, Summer 2003.
  Person responsible in the Centre: Dr. Anna Maciołek.
- WP4: 1st International Workshop on 'Surface Modification for Chemical and Biochemical Sensing' SMCBS'2003, Białowieża, Poland, November 13-16, 2003. 2nd International Workshop on 'Surface Modification for Chemical and Biochemical Sensing' SMCBS'2005, Kazimierz Dolny, Poland, November 6-10, 2005.
  Person responsible in the Centre: Prof. Włodzimierz Kutner.
- WP5: Strengthening of co-operation on standardisation and quantification of surface science techniques. Person responsible in the Centre: Prof. Aleksander Jabłoński.
- WP6: Training of scientists and Ph.D. students in surface science methods.
  Persons responsible in the Centre: Dr. Iwona Flis-Kabulska Prof. Zbigniew Karpiński and Prof. Jerzy Pielaszek Dr. Beata Lesiak-Orłowska, Dr. Robert Nowakowski.
- WP7: Management and co-ordination of the Centre.
  Person responsible in the Centre: Prof. Janusz Flis.

International attraction

Research results are presented at various national and international scientific meetings. Then, they are published in scientific specialty journals or as monographs; some results are directly applied in industrial processes. Interdisciplinary attitude of the Institute to science requires a large co-operation between the Departments and other research centres. Active participation of the scientists from the Institute in international events attracts other centres to mutual co-operation.

Every year over a hundred scientists from Europe, America and Asia visit our Institute. Majority of visits is connected with participation of our guests in conferences and symposia. Many scientists from European and other countries collaborate with researchers of the Institute by giving lectures and by being engaged in the join research. Our top quality commercial and unique home-built instrumentation encourages foreign researchers to perform experiments in our laboratories.

The SURPHARE Centre of Excellence is engaged in the long-term co-operation with scientific institutions of Europe and other continents. The researchers of the Centre are involved in eleven international and national research projects. Moreover, they collaborate with industrial enterprises, such as power plants and refineries. Competence of the scientists and fluency in foreign languages (English is spoken by most researchers while German, French and Russian are less common) enable the Institute researchers to co-operate with their peers in foreign partner institutions.

Programme

Sunday, 6 November

Registration and lunch

IChF PAN, Warsaw, Kasprzaka 44/52
Sunday afternoon, 6 November, 12:00

Travel to Kazimierz Dolny by bus

Sunday afternoon, 6 November, 14:00

Get-together party

Sunday evening, 6 November, 18:30

Monday, 7 November

Breakfast

Monday morning, 7 November, 8:00

SESSION 1

Chairpersons: Lo Gorton, Zbigniew J. Stojek
Monday morning, 7 November, 9:00

9:00 Tutorial lecture

Conducting polymers as model biological membranes

Andrzej Lewenstam

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Conducting polymers (CPs) films, poly(3,4-ethylene- dioxythiophene) (PEDOT) and poly(pyrrole), doped with adenosine triphosphate, heparin, asparagine or glutamine and made sensitive to calcium and magnesium are used to study the influence of a competitive magnesium and calcium ion-exchange on a membrane potential formation.

The films are analyzed by X-ray Photoelectron Spectroscopy (XPS) and Energy Dispersive Analysis of X-rays (EDAX) to show how their topography and morphology is influenced by the conditions during electrodeposition and post-deposition soaking. Atomic Force Microscopy (AFM) and Scanning Electron Microscopy (SEM) are used to prove that the topography and morphology of the films determines the quality of their open-circuit (potentiometric) response. More smooth and less rough films result in better potentiometric characteristics, particularly in a faster response, which is a prerequisite for using the CPs films as model biological membranes.

Membrane potential transients provoked by a competitive calcium and magnesium ion-exchange at the sites (dopants) dispersed in the films, and the mechanism of activation of calcium channels blocked by magnesium-site interaction are investigated. Theoretical interpretation in time domain is obtained by the numerical resolution of Nernst-Planck-Poisson (NPP) coupled equations or simpler diffusion-layer model, deduced from the NPP.

9:40 Keynote lecture

Nanoscale dielectrophoresis as a tool for studies of single biomolecules in aqueous environment

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The discovery of dielectrophoresis (DEP) goes all the way back to at least 600 B.C., when Thales of Miletus in Turkey observed that rubbed amber attracted small particles of fluff. The induced dipole in the fluff particles was acted on by the electric fields, attracting it to the charged amber. This effect can be referred to as (dc) positive dielectrophoresis. One of the most promising applications of dielectrophoresis in nanotechnology is the possible electronic manipulation of individual molecules. Herein a 100 nm scale water transistor is described. The structure can both capture proteins and measure the proteolytic activity of enzymes such as glucose oxidase.

This nanoscale structure has earlier been characterized [1]. Herein enzymes are introduced to the structure in order to push the limits for new types of enzyme nano sized biosensors. A nano sized enzyme biosensor exhibits much lower reaction volumes than conventional technologies. Electron-beam lithography will be a promising rout to biosensor fabrication. This novel device is demonstrated on glucose, using glucoseoxidase, however it opens up the possibility to study any proteolytic enzymereaction in sub femtoliter reaction volumes.

[1] Z. G. Chiragwandi, O. Nur, M. Willander, N. Calander, *Appl. Phys. Lett.*, **83** (2003) 5310.

10:00 Keynote lecture

Modification of the transducer - a key step for achieving improved analytical parameters of conducting polymer based all-solid-state ion-selective electrodes

Agata J. Michalska, Krzysztof Maksymiuk

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All-solid-state ion-selective electrodes (ASS-ISEs) often apply conducting polymers as ion-to-electron transducers, placed between ionic conductor - ion-sensing membrane and electron conductor - supporting material. This choice is justified by superior stability of the analytical signal - potential measured, a fact confirmed for numerous sensors of this type.

Recent advances in the field of internal solution ISEs, especially lowering the detection limit and improving obtainable selectivity coefficients set new standards of performance also for ASS-ISEs, with ultimate goal of making them competitive in terms of analytical parameters with the up today tailored internal solution ISEs.

The important step was identification of the processes related to the conducting polymer phase, influencing the detection limits and selectivities of ASS-ISEs. Thus, successful elimination of undesired effects was possible and yields all-solid-state sensors of performance well comparable with tailored internal solution electrodes of low detection limit and high selectivity. Various methods can be applied to obtain improved all-solid-state potentiometric sensors: these are related both to sensor pretreatment, method of measurement as well as modification of the transducer and choice of membrane ion-selective membrane material applied. All methods prove to be successful and result in more favorable sensors analytical parameters.

10:20 Short communication

Electrochemical reactivity of proteins immobilised in mesoporous titania

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Thin mesoporous films of TiO_2 (anatase) particles (or similar types of nanoparticles) are assembled at electrode surfaces via layer-by-layer deposition techniques. Depending on the particle size and the number of deposition cycles, functionalized films of typically 30 to 300 nm thickness are formed and characterised by atomic force microscopy (AFM), scanning electron microscopy (SEM), voltammetric, and impedance methods.

Depending on the type of mesoporous membrane and the adsorbed redox system, electrochemical processes close to the electrode surface (Process 1) or throughout the mesoporous film (Process 2) are observed. In some cases Process 2 is inhibited by slow electron conduction through TiO_2 and in the presence of a soluble redox mediator strong amplification of voltammetric signals is observed.

Coffee break/Poster session

Monday morning, 7 November, 10:35

10:35 Poster 1

Mixed self-assembled monlayers (SAMs) for immunosensor construction

Morsaline Billah[1], Henry Hays[1], Sophie Weiss[1], Tim Gibson[2], Paul A. Millner[1]

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Immunosensors detect and quantify analytes based on binding related to changes in mass, resistance or capacitance at sensor surfaces. Key to sensor development is the ability to immobilise antibodies and biological recognition elements, via procedures that allow binding affinity to be retained. Mixed self-assembled monolayers (SAMs) comprising long chain alkanethiols i.e. 16-mercaptohexadecanoic acid (MHDA) on gold with incorporated affinity lipids, i.e. 1,2-Dipalmitoyl-*sn*-Glycero-3-Phosphoethanolamine-N-Cap Biotinyl (Biotinyl cap PE) have been developed to allow antibody tethering via the biotin/Neutravidin interaction. Alternatively, mixed SAMs of 16-mercaptohexadecanoic acid (MHDA) and intercalated 1,2-Dioleoyl-*sn*-Glycero-3-[(N-(5-amino-1-carboxypentyl) iminodiacetic acid) succinyl] (Nickel salt) (DOGS-NTA) were used to immobilise His_6-tagged recombinant proteins. Large analytes i.e. haemoglobin (Hb), myoglobin (Mb), prostate specific antigen (PSA) and small analytes, i.e. atrazine and digoxin were studied.

Partially constructed and fully functional immunosensors were characterized via physical techniques including quartz crystal microbalance (QCM) and atomic force microscopy (AFM). Sensor assembly and binding affinity of immobilised antibodies have been independently determined using $[^{125}I]$-tagged components. Finally, the electrochemical properties of semi-constructed and functional electrodes have been analysed by impedance based measurements at 300 mV (10mV amplitude) at a frequency range between 100kKHz - 0.1 Hz and using a potassium hexacyanoferrate trihydrate mediator.

Furthermore, phospholipids on mercury (Hg) passivation using polyethylene glycol (PEG) incorporation in mixed SAM system was studied to suppress the non-specific binding of protein to the surface.

10:35 Poster 2

The electrochemistry of a his-tagged microperoxidase assembled onto gold electrodes

Vasile Coman[1], Lo Gorton[1], Claes Von Wachenfeldt[2], Cecilia Hägerhäll[3]

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Microperoxidases are used as model compounds for hemoproteins and have potential applications as labels for antibodies, as electron transfer mediators, in biosensors and biofuel cells. Their relative structural simplicity yet possessing peroxidase activity, and the presence of chemical functions suited for covalent coupling or modification are the advantages for practical applications in the biological and medical research fields.

Direct electron transfer (DET) between an immobilized redox enzyme and an electrode is one of the central themes in bioelectrochemistry, being one of the most interesting transduction processes for the development of fast responding amperometric biosensors. In order to obtain a high-speed, efficient DET one has to take into consideration the importance of interfacial interactions, the structure of biolayers and the distance between the active site of the biomolecule and the electrode.

As a continuation of our previous work on the electrochemistry of microperoxidase-11 immobilized onto gold electrodes in different ways, we here report on the electrochemistry of an artificial his-tagged microperoxidase (MP-86), obtained *in vivo*, using the cytochrome *c* secretion and maturation machinery of *E. coli*. The artificial heme-containing oligopeptide possesses a classical heme *c* binding signature sequence followed by a 6xhis-tag. The his-tag facilitates purification and stabilizes the heme group but also enables specific immobilization of the polypeptide onto solid surfaces.

Cyclic voltammetry and flow injection amperometry are the basic electrochemical techniques used in this study. The immobilized microperoxidase assembled as a monolayer onto gold undergoes a rapid, reversible electron transfer. The 6xhis-tag favors both adsorption onto naked gold electrodes and specific immobilization using Ni-NTA technology, conserving a short distance between the heme group and the electrode. In order to demonstrate the peroxidase activity of MP-86, electrocatalytic reduction of hydrogen peroxide in a flow system was also performed.

Acknowledgement. This work was supported by the European Commission (MEST-CT-2004-514743) and The Swedish Research Council.

10:35 Poster 3

Amperometric determinations of reducing enzyme activities in living whole S. cerevisiae cells immobilized on micro-band electrodes

Jenny Emnéus[1], Christer Spegel[2], Arto Heiskanen[1], Ted Johansson[3], Marie-Francoise Gorwa-Grauslund[3], Milena Koudelka[4], Tautgirdas Ruzgas[5]

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A mediated amperometric method has been utilized for the determination of menadione (M) reducing enzyme activity as well as diketone reductase (DkR) activity in living, genetically modified, *S. cerevisiae cells*. The electrochemical results corresponded well to in vitro spectrophotometrically determined reductase activities. The amperometric method is based on M, a lipophilic quinone able to diffuse freely through the plasma membrane, and accept electrons from intracellular NAD(P)H dependent enzymes. The reduced form of M, menadiol, is extracellulary oxidised by ferricyanide, which in turn is oxidised at the platinum microband electrode. This process results in a current proportional to the activity of the intracellular M reducing enzymes [1].

It was found that the amplitude of the yeast-catalysed amperometric signal was dependent on the metabolic pathway supplying the NAD(P)H, in the order; ethanol oxidation pathway(EOP) > pentose phosphate pathway (PPP) > glycolytic pathway (GP). An optimum in the apparent electrochemical Michaelis-Menten (MM) constants for intracellular DkR was found for an M concentration of 200 mM, *i.e.* a minimum in the apparent K_M and optimum in apparent i_{max}.

[1] A. Heiskanen, J. Yakovleva, C. Spégel, R. Taboryski, M. Koudelka-Hep, J, Emnéus, T, Ruzgas, *Electrochem. Com.*, **6** (2004) 219.

10:35 Poster 4

Properties of monolayers of azocrown ethers: Ph -tunable equilibria for compounds with substituents containing mobile protons

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The crown ethers with electro- and photoactive azo moieties containing -OH, or COOH groups show unique effect of pH switched on/off presence of the azo form. It is well known that electrode processes of azocompounds are pH dependent but we show here that they are also strongly influenced by substituents containing mobile protons such as in -COOH groups. Azocrown ether with N-acetyl-histidine moiety in the molecule gave different Raman spectra compared to those of the imidazole azocrown ether, however, only in acidified solutions. . In alkaline medium the spectra were similar.

The differences in the electrochemical reduction of the imidazole and histidine derivatives in strongly acidified solutions reflect the interference of chemical reduction. It is catalyzed by mobile protons of the -COOH group present in the histidine derivative, leading further to the formation of the hydrazine form. Thus, for the N-acetylhistidine azomacrocyle similar behaviour is revealed to that of azophenol compounds which are known to exist in quinone-hydrazone tautomeric equilibria.

10:35 Poster 5

Designing of cathodes modified with enzymes for the biofuel cell applications

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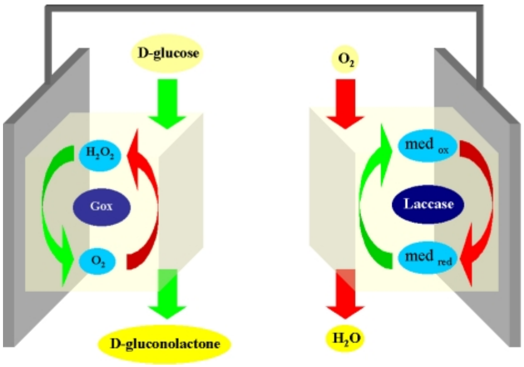
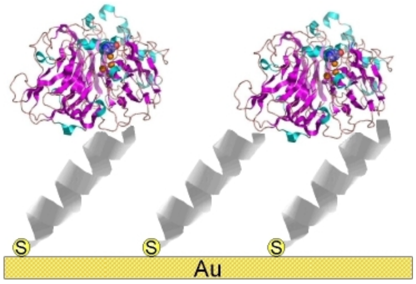
Biocatalysts are employed in biofuel cells for the conversion of chemical energy to electrical energy. The enzymes can be used in one of two ways. Either the biocatalysts can generate the fuel substrates for the cell by biocatalytic transformations or metabolic processes, or the biocatalysts may participate in the electron transfer chain between the fuel substrates and the electrode surfaces. Unfortunately, most of redox enzymes do not take part in direct electron transfer with the conductive supports, and therefore a variety of electron mediators are used for the electrical wiring of the biocatalyst to the electrode.



An important condition for the progress in biotechnology is to understand the electrochemical reactions of redox proteins and to control their interactions with gold substrates. Gold is often used as the electrode material due to its inertness. One of the crucial steps in the development of electrodes for biofuel cells is the appropriate immobilization of the enzyme on gold, since its biological activity as well as electrical contact have to be preserved.

Laccase (EC 1.10.3.2) is a blue multi-copper enzyme which catalyses the oxidation of a variety of phenolic and amine compounds simultaneously with the four-electron reduction of molecular oxygen to water. The enzyme active site contains four copper atoms of types I, II, and III, which play different roles in the enzymatic process. Substrates are oxidized near the T1 site, and the electrons are transferred to the T2/T3 cluster, where molecular oxygen is reduced.

We employed several strategies to immobilize laccase on gold, such as electrostatic adsorption on gold clusters, entrappment of the enzyme in poly(N-isopropylacrylamide) gel (NIPA) and covalent attachment to self-assembled organothiols monolayers (SAMs).



In case of NIPA gel matrix the immobilized laccase showed electroactivity at 0.360 V vs. NHE, however, upon binding to 11-mercaptoundecanoic acid (MUA) the Cu center is electroactive at a more positive potential (590 V vs. NHE). The catalytic current of oxygen reduction was observed in case of Au cluster/laccase modified electrode. For the MUA/laccase electrode, for both mediatorless and mediated with 1,1'-ferrocenedimethanol processes, the catalytic oxygen reduction was observed. Hence, best results obtained so far are those based on 11-mercaptoundecanoic acid (MUA) as the molecular bridge.

10:35 Poster 6

Electrochemical restructuring o polyaniline films in the presence of catechols

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It is shown that electrochemical cycling of polyaniline modified electrodes in acidic electrolytes containing dissolved catechol(s) results in covalent attachement of this species to polyaniline. Electrochemical properties of polyaniline are significantly altered and a new voltammetric peaks appear that are not assigned to parent polyaniline nor catechol (Fig 1).

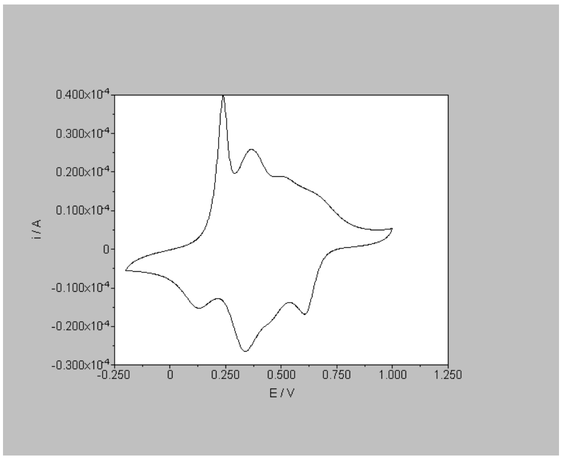

Fig. 1. Cyclic voltammogram of catechol-restructured polyaniline film in 0.1M H_2SO_4

The proposed mechanism involves nucleophilic addition of polyaniline to oxidized form of catechol (o-quinone). When sulfonated catechol is generated by the oxidation of guaiacol sulfonic acid on polyaniline film it also attaches to the polymer to form a new self-doped composite retaining its electrochemical activity in neutral electrolytes. Morover it exhibits persistent electrochemical activity toward the oxidation of ascorbic acid.

10:35 Poster 7

Lipidic cubic phase for immobilising enzymes on electrodes

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Lipidic cubic phase is used for the immmobilization of enzymes on the electrode surface. The lipidic cubic phase can be characterised as a curved, non-intersecting bilayer with two unconncted water channels. Monoolein (MO) is an example of a lipid forming such a phase. At hydration over 20%, the cubic phase is stable in aqueous solutions. MO was mixed with water in 64/36% ratio.

In cubic phase, the diffusion of both hydrophobic and hydrophilic compounds can take place. Due to high viscosity and stability in aqueous solutions cubic phases are good matrices for immobilizng enzymes on electrodes and providing electric contact with electrode surface. Additionally, they stabilise membrane enzymes and protect them against denaturation.

Glucose oxidase, pyranose oxidase and laccase were immobilized in water-monoolein cubic phase. Their activity was monitored using cyclic voltammetry. Catalytic oxidation of *β*-D-glucose at the platinum electrode covered with cubic phase containing glucose or pyranose oxidases leads to hydrogen peroxide. Its oxidation was detected amperometrically. Reduction of oxygen was catalyzed using laccase as the biocatalyst.

The mediators can diffusionally shuttle electrons between the electrode and enzymes and improve the electric contact between them. Hexaammineruthenium(II) chloride and ferrocenecarboxylic acid were used as mediators for processes catalyzed by glucose oxidase and pyranose oxidase. ABTS and hydroquinone were the mediators for laccase catalyzed reduction of oxygen.

10:35 Poster 8

Prussian Blue based nano-electrode arrays for detection of H_2O_2 with advanced analytical performances.

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Monitoring of low levels of hydrogen peroxide is of great importance for modern medicine, environmental control and various branches of industry. The most progressive method of H_2O_2 monitoring is its amperometric detection on electrode, modified by Prussian Blue (PB). PB modified electrodes allow low-potential detection of H_2O_2 down to 10^{-7}M [1].

Despite the possibility to detect hydrogen peroxide down to 10^{-7} M achieved, both clinical diagnostics and environmental control in certain cases require monitoring of lower H_2O_2

levels. The decreased detection limit is possible with the use of micro-electrodes instead of conventional ones. We have reported before on the possibility for nanostructuring of PB by its electrodeposition through liquid crystal template for improving of analytical performances. Prussian Blue is a superior electrocatalyst of hydrogen peroxide reduction, where as graphite materials possess in this reaction only a minor activity. Hence, the resulting nano-structured Prussian Blue on carbon can be considered as nano-electrode array in relation to H_2O_2 electrochemical reduction [2].

Now we repot on the possibility to simplify the PB nanostructuring procedure. It may be done by electrodeposition of nanostructured PB films without using template. Analytical performances of the resulting PB based nanoelectrode arrays have been studied in course of hydrogen peroxide detection in FIA mode. The value of sensitivity for obtained sensors was 0.2 $AM^{-1}cm^{-2}$, which is two times more than for electrodes modified by PB electrodeposited through liquid crystal template. Detection limit was 10^{-8}M and a linear calibration range was extending over six orders of magnitude of H_2O_2 concentrations, which are the most advantageous analytical performances in hydrogen peroxide electroanalysis nowadays.

[1] A. A. Karyakin, *Electroanalysis*, **13** (2001) 813.
[2] A. A. Karyakin, E. A. Puganova, I. A. Budashov, I. N. Kurochkin, E. E. Karyakina, V. A. Levchenko, V. N. Matveyenko, S. D. Varfolomeyev*, Anal. Chem.,***76** (2004) 474.

10:35 Poster 9

Heavy metals sorption in lichen cationactive layer

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Bioindicative properties of lichens were observed by William Nylander as early as in the 19th century. He noticed that some lichen species began to die out in Paris agglomeration and associated this fact with the increasing air pollution. Lichens, owing to their anatomy and peculiar mode of nutrition, accumulate substances contained in the air and precipitation. Air pollutants, depending on their type and concentration, build in the lichen texture and cause physiological and morphological changes or lead even to thallus destruction.

Studies described in the literature indicate that there is an interrelation between pollutant content of lichen thallus and its concentration in the environment. Problems related to study methodology standardisation stem from different climate conditions, type and composition of substratum on which lichens grow, as well as from airborne aerosol composition on studied areas. So far some have been studies conducted on the impact of these factors on sorption intensity. Heavy metals are the most frequently studied environment pollutants, as their presence in the natural environment indicates the pollution origin and intensity, as well as pollutant spreading directions.

Previous studies demonstrate that heavy metal sorption in lichens occurs as the result of heterophase ion exchange between lichen cationactive layer and precipitation with which lichens contact. Building in of cations into lichen thallus texture is the parallel or secondary process. The aim of present studies is to investigate the impact of abiotic factors on coefficients of heavy metal cation distribution between the environment and lichen thallus. The impact of precipitation composition and temperature on the process course was examined among other things. Studied processes of sorption of heavy metals from solutions, in which lichens were dipped, were described by means of mathematic models of Langmuir and Freundlich isotherms.

10:35 Poster 10

Amperometric determination of formaldehyde by the use of the yeast Hansenula polymorpha as a bioselective element

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Formaldehyde is one of the most important chemicals widely used in industry. Its classification as a mutagen and human carcinogen has motivated a strong need for formaldehyde control in environment and food. In order to measure the concentration of formaldehyde, a highly selective and stable biosensor is required, which can be based on the yeast cells capable of metabolizing formaldehyde. In the present study we tested the possibility to use living and permeabilized cells of the yeast *Hansenula polymorpha* for construction of amperometric biosensors selective to formaldehyde. The following experimental methods have been used: (i) measurement of the current produced by the cells in the presence of mediators based on the methodologies proposed in [1,2]; and (ii) measurement of oxygen consumption with a biosensor based on immobilized yeast cells. (i) It was found that both type of yeast cells produced rather high current in the presence of mediators. However, the current did not depend quantitatively on the formaldehyde concentration in the case of permeabilized cells. It was shown that digitonin used for the permeabilisation produces distortions of some essential membrane structures responsible for the normal work of the respiratory electron transport chain. Indeed, a biosensor based on living yeast cells was designed and its basic parameters, such as detection limit (0.5 mM), linear dinamic range (1-10 mM), long-term and operation stabilities were determined. (iii) The device with immobilized permeabilized yeast cells allowed determination of formaldehyde concentration only up to 4 mM at 24ºC. Similar results were obtained in a previously reported work [3] by using the pH-FET-based potentiometric biosensors. We can suggest that the rapid limitation of output signal with formaldehyde concentration is caused by regulatory properties of the complex AOX/membrane. A marked decrease in respiratory activity of permeabilised cells was observed at formaldehyde concentrations greater than 4 mM, probably as a result of a deterious effect of formaldehyde on the yeast cells.

[1] B.A. Kuznetsov et al., *Bioelectrochem.*, **64** (2004) 125.
[2] A. Heiskanen et al., *Electrochem. Commun.*, **6** (2004) 219.
[3] Y. I. Korpan et al., *Biosens. Bioelectron.*, **15** (2000) 77.

Acknowledgement. The work was supported by INTAS Open Call project No. 03-51-6278

10:35 Poster 11

Electron transport through alkanethiolate films decorated with monolayer-protected gold clusters

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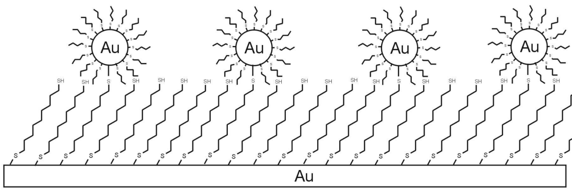
Modifying layer containing gold clusters on a monolayer of organothiol transfers charge to the redox couple in the solution more efficiently than without nanoclusters [Scheme 1].

The clusters bound to the electrode by means of 1,9-nonanedithiol (NDT) are more uniformly distributed on the monolayer and their efficiency in transferring electrons between the electrode and $[\mathrm{Fe(CN)}_6]^{4-/3-}$ couple in the solution is better than when they are bound by means of weak van der Waals interactions only (as in case of 1-decanethiol (DT)). With increasing adsorption time in the solution of clusters, the capacitance of the cluster decorated electrode significantly increases compared to its constant value after prolonged immersion in pure toluene. This difference is explained assuming that gold clusters adsorb on the monolayer modified surface and act as an array of capacitors increasing in number.



Scheme 1. Structure of electrode modified with gold nanoparticles supported on gold electrode by means of organothiol monolayer.

The 4-hydroxythiophenol monolayer modified gold clusters attached to electrodes by means of dithiol molecules transfer charge to the DOPA (3,4-dihydroxyphenylalanine) and ascorbic acid (AA) molecule in the solution more efficiently than when the same 1,9-nonanedithiol or 4-hydroxythiophenol are assembled in form of a monolayer directly on the gold substrate. Probably, the clusters attached to the monolayer on the electrode surface play here the role of electron accepting units, and charge during the potential scan. This would allow them to act as individual microelectrodes in the process of AA and DOPA oxidation.

10:35 Poster 12

Towards the protein phosphatase-based biosensors for microcystins detection

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Microcystins are cyclic heptapeptides, composed of five permanently occurring and two variable amino acids. The toxicity of microcystins is associated to the inhibition of the serie/threonine protein phosphatases type 1 (PP1) and 2A (PP2A), enzymem that play an important role in the dephosphorylation of intracellular proteins. Thein inhibitionh in the liver implies hyperphosphorylation of the cytoskeletal filaments, hepatocyte determination and subsequent tumor formation. In order to assure the water quality and the public health, the WHO (World Health Organization) has recommended a maximum level of 1 µg/L of most commonly fund microcystin-LR in drinking water. The strong toxicity of microcystins makes necessary the development of fast, sensitive and reliable methods to detect them. The enzymatic approach based on the PP inhibition informs about the toxicology and is highly sensitive. Electrochemistry has been widely used in biosensors because but it has not been exploited neither for the detection of protein phosphatase inhibition by microcystins. We propose new strategy for the electrochemical monitoring of microcystin in water. The enzymatic approach is based on the inhibition of protein phosphatase (type 2A) by microcystins. The enzyme has been immobilised on the screen-printed working electrode surface by encapsulation with polyvinyl alcohol bearing styrylpyridium groups (PVA-SbQ). Immobilisation conditions have been optimised by colorimetry. The electrochemical transduction has been achived by using a new designed enzyme substrate, catechyl monophosphate, wich is electrochemically active only after dephosphorylation by the non-inhibited enzyme. This bioelectrochemical sensing strategy have led to development of amperometric biosensors for water quality assessment.

10:35 Poster 13

Electrostatic immobilisation strategies for attachment of enzymes and antibodies to biosensor surfaces.

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In biosensor construction, immobilisation of the biomolecule acting as the sensing agent represents a key step. Tethering of the sensor molecule, which is in most cases an enzyme or antibody must be performed in such a way as to enable sufficient binding or catalytic activity to be retained in order to provide a reasonable signal.

With printed carbon electrodes, the availability of active surface groups to permit easy coupling of the sensor biomolecule is normally a limitation. We have introduced additional amine moieties to the carbon surface via electrochemical reduction of aryl nitro groups after inital derivatization with nitrobenzenediazonium. These amines can be used for direct coupling to sensor biomolecules or subject to sequential grafting of polyethyleimine/ terepthladehyde layers which can be used for electrostatic tethering. Using these approaches we have consrtucted acetylcholine esterase (AchE) based organophosphate sensors. Such sensors operate amperometrically, via organophosphate inhibition of the AChE activity towards acetylthiocholine.

In matrix based immunosensors, pyrrole or aniline monomers were electropolymerised onto gold electrodes. This process can be followed both electrochemically and via quartz crystal micrbalance measurements. The resulting surface is positive at neutral pH and will bind IgG and other antibodies to produce a functional immunosensor. However, multiple sequential additions of the polyanion polstyrene sulphonate (PSS) and the polycation polydiallyldimethyl ammonium chloride (PDADM) improve both the kinetics of antibody capture and also antibody loading onto the sensor surface. Digoxin sensors which are interrogated impedimetrically have been produced in this way.

Finally, unmodified polypyrrole layers have been found to be relatively unstable to wet storage. Increases in stability can be brought about by alkanethiol masking of available sites on the gold surface following deposition of the polypyrrole layer. In general, the use of polyelectrolyte layers produces surface which permits, rapid, gentle and tight immobilisation of biosensor proteins and permits easy self nanoscale self assembly of the biosensor surface.

10:35 Poster 14

Enantioselective amperometric screen-printed biosensor with d-amino acid oxidase for determination of d-alanine in food

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D-alanine is considered as important marker of bacterial contamination of food products. Usually it is determined using various modes of HPLC with chiral selectivity (see e.g. [1]) or GC after suitable derivatization. The aim of this work was to investigate the possibility of design of screen-printed amperometric biosensors using D-amino acid oxidase (DAAOx) and examine its selectivity for determination of D-alanine. DAAOx oxidizes D-alanine to puryvate and produces hydrogen peroxide from the oxygen reduction. It results in regeneration of enzyme to oxidized form. Hydrogen peroxide is a source of analytical signal, based on transition of the Prussian Blue from reduced to oxidized form.

The three electrode screen-printed sensors used in this study were produced in Department of Chemistry, University of Florence, Italy. The graphite working electrode was first pretreated by anodic polarization in phosphate buffer and then modified by Prussian Blue, precipitated in-situ from mixture of hexacyanoferrate (III) and ferric chloride on the electrode surface [2]. The carefully dried surface of modified working electrode was covered with cation exchange protective layer produced by evaporation of Nafion solution. Then DAAOx was immobilized on this surface by cross-linking with bovine serum albumin and choline oxidase using glutaraldehyde. It was found necessary to purify the commercial preparation of DAAOx by dialysis in order to remove Tris salts interfering in immobilization. The observed higher stability of biosensor response in buffer containing FAD indicates that cofactor is being removed during dialysis or storage of biosensor in solution. The biosensor optimization included selection of the suitable buffer, pH of measurements, composition of the enzyme layer and the way of its preparation and storage. Sensitivity and the life time has been also examined.

A fast and linear response of developed biosensor was observed in static measurements for D-alanine in the concentration range from 5 to 200 mM. The excellent enantioselectivity towards D-amino acids was found. The complex matrix of natural milk samples had no influence on the response of biosensor.

Multiwalled carbon nanotubes have been used for modification of working electrode. The effect of modification for the sensitivity of biosensors has been checked.

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10:35 Poster 15

Fine-tuning of properties of bismacrocyclic dinuclear cyclidene receptors by N-methylation.

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By changing properties of components of mechanically bonded molecules, one can variate properties of resulting complex structures. Such a tuning of properties can be achieved by severe measures as, for example, replacement of the metal ions, elongation of the aliphatic linkers or change of macrocycles. On the other hand, more subtle changes of properties can be introduced by proper chemical modifications of particular positions of the parent systems. We present the role of N-methylation in controlling the properties of bismacrocyclic substrates used for the construction of more complex molecules.

N-methylated bismacrocyclic Cu and Ni complexes were synthesized and structurally characterised in the solid state. Their properties in the solution were analysed using NMR, ESR spectroscopies and electrochemical methods. Face-to-face biscyclidenes linked via polymethylene chains form rectangular box-like cations. These moieties can host some small guests molecules (water, p-electron donating compounds) and are stabilised by a shell of neighbouring couterions.

For the bismacrocyclic dinuclear complexes containing two nickel or two copper ions, the intramolecular interactions between the metallic centres are strengthened through methylation of the macrocyclic components comparing to the non-methylated species.

Methylation weakens electron acceptor properties of the complexes leading to less effective binding of the p-electron donating guests. It also increases the stability of the lower oxidation states. In the case of the copper complexes both Cu(II)/Cu(I) and Cu(II)/Cu(III) reversible 1 electron transfers are seen in the voltammograms. These changes in properties are interpreted as consequences of steric repulsion between the methyl substituents and the macrocyclic ring.

10:35 Poster 16

Electrochemical characteristics and interaction with anions of homo- and heterodinuclear biscyclidene Ni(II) and Cu(II) complexes bridged by two di-aza-18-crown-6 ethers

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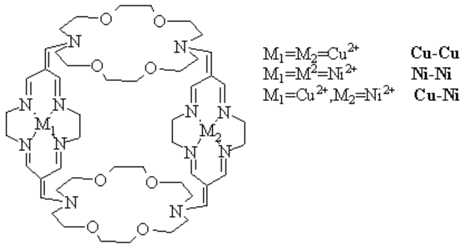
In our previous work we studied mono-nuclear Cu(II) and Ni(II) cyclidene complexes functionalised with one or two aza-crown ethers [1].

The aim of present studies was the synthesis and physicochemical characterisation of dinuclear complexes containing two cyclidene metal ion coordinating units, linked by two diaza-18-crown-6 ethers. Two the of studied complexes were homodinuclear and one of them was heterodinuclear containing copper(II) and nickel(II) cyclidens in the same molecule. Structure of studied complexes is presented in Scheme 1.



Scheme 1

In our studies, we focused on investigation of electrochemical properties of bismacrocyclic compounds. Especially, interaction between metal centers and behaviour in the presence of small molecules, which could be accommodated in the cavity, was interesting for us. The influence of the presence of anionic guests of different shape and size on electrochemical properties of studied complexes will be presented.

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Makal, K. Woźniak, *Eur. J. Inorg. Chem.*, (2004) 3335.

10:35 Poster 17

Molecularly imprinted polymers by gamma-irradiation polymerization

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Molecularly imprinted polymers (MIPs) are relatively a new class of selective sorbents. Many applications of these materials have been reported such as stationary phases for HPLC, TLC or SPE, artificial antibodies in binding assays. They have also been successfully used as sensor materials in chemical sensors, due to their high selectivity.

The essence of their preparation is that the target molecule (template) is present in a polymerization mixture where one of the monomers is able to bind to it through a functional group (functional monomer) and another monomer (crosslinker) fixes this complex by creating a polymer network. After polymerization the template is removed leaving behind functional groups in a specific spatial arrangement that can rebind the target analyte.

There are two basic methods for the creation of the template functional monomer complex. One of them is covalent imprinting where the template is covalently bound to the monomer before polymerization and the resulting relatively weak covalent bonds are disrupted when the polymerization is finished. The other, more versatile and simpler method is non-covalent imprinting. In this case the template binds to the monomer in a self-assembly process with van der Waals forces in the pre-polymerization mixture. In this case the resulting polymer usually have lower selectivity, but the MIP preparation and template removal is much easier, and the method is aplicable to a lot more target compounds.

Non-covalently imprinted polymers are usually synthetized by thermally or UV initiated free radical polymerization. The advantage of thermal polymerization lies in its simplicity and low cost, however the high temperature which is demanded by the initiator molecule does not favour template monomer complexation. The temperature of the UV polymerization can be adjusted freely, but it is more complicated and the homogeneous illumination of the polymerization mixture is problematic. The disadvantage of both methods is that an initiator molecule is needed to start the reaction the solubility of which can cause problems.

Ionizing radiation like γ radiation is also capable of initiating polymerization from appropriate monomers. This method has the advantage of generating radicals directly on the monomer, thereby avoiding the use of any initiator. The temperature can be set as wished, even large samples can be homogeneously irradiated.

In our work poly(metacrylamide-co-ethyleneglycol-dimethacrylate) polymers have been imprinted with phenytoin using γ irradiation. The irradiation parameters like irradiation dose, dose rate and polymerization temperature have been optimized. The polymers have been tested in batch adsorption exepriments and compared to thermally polymerized MIPs.

10:35 Poster 18

Some electrochemical properties of laccase immobilised on the Au, IrO_x, or C_{60}-Pd polymer electrode supports

Katarzyna Klimek[1,2], Wlodzimierz Kutner[3], Andrzej Czerwiński[1,2], Hanna Elzanowska[1], Lo Gorton[4]

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In the experiments which aimed at unravelling electrochemical activity of laccase (*Trametes hirsuta*) redox centres, the enzyme has been adsorbed and its properties studied on the Au, IrO_x, or C_{60}-Pd electrode support in McIlvaine's buffer at pH»4.0, using cyclic (CV) and square wave (SW) voltammetry. In case of the Au electrode, only one redox couple was detected for laccase at ca. 0.30 V vs. SCE. However, a redox signal of this couple was split into three peaks at ca. 0.25 V, 0.40 V, and 0.55 V in the presence of catechol, i.e. one of the enzyme substrates that is not oxidized below 0.60 V. Presumably, these peaks, which increased after co-immobilization of Cu(II) ions within the enzyme film, corresponded to the oxidation of various copper centres of laccase. The heights of these peaks depended on both catechol and oxygen concentration in solution. It appeared that the laccase redox centres were inter-exchangeable when the enzyme was allowed to react with both reagents. For instance, after the addition of catechol, which is typically oxidized by laccase causing its reduction, the enzyme anodic peak at 0.40 V increased, while the anodic peak at 0.25 V decreased in the presence of oxygen. Moreover, catechol has been used as a mediator to study oxygen reduction catalysed by laccase. For these investigations, laccase was immobilized on a nanoparticulate iridium oxide (IrO_x) film and also on a Pd-containing C_{60}-Pd polymer film. The oxygen reduction potential on laccase-coated IrO_x was close to +0.1 V, compared to -0.1 V on Au. However, the reaction mechanism involving oxygen reduction to water rather than to hydrogen peroxide still remains to be proven. The most promising results have been obtained with the use of the C_{60} polymer film. This film was prepared by CV electropolymerisation of C_{60} in the presence of Pd(II) acetate trimer using a mixed solvent of 4:1 (*v*:*v*) toluene and acetonitrile. The C_{60}-Pd polymer features a pair of anodic and cathodic reversible CV peaks at ca. 0.25 V. In the absence of the catechol mediator, reduction of oxygen is enhanced only slightly by the adsorbed laccase. However, the addition of catechol, which reveals a quasi-reversible CV anodic and cathodic peak pair at ca. 0.35 V, results in the mediated reduction of oxygen by laccase at the reduction potential of this mediator.

10:35 Poster 19

Electrochemical quartz crystal microbalance study of the avidin and albumin adsorption under flow injection analysis conditions

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In humans, albumin is responsible, among others, for isolation and removal of foreign intruding solid objects. Thus, implants can be clogged and rejected as well. Therefore, we have undertaken an effort to find conditions, in vitro, under which adsorption of albumin on a solid substrate surface is prevented. Virtually, albumin does not interact with avidin. Therefore, an idea was explored to coat, first, the studied solid surface with avidin in hope that albumin will not adsorb onto it afterwards. For effective adsorption of avidin, we used an avidin-biotin immobilization procedure and monitored this adsorption by using piezoelectric microgravimetry with an electrochemical quartz crystal microbalance (EQCM) under batch conditions. We used a matrix of a conducting polymer film for biotin immobilization by ion exchange. That is, the surface of a gold electrode of the 5 MHz resonant frequency quartz crystal piezoelectric transducer was coated with a polypyrrole film doped with d-botin 4-aminobenzoic acid sodium salt. The film was prepared by electropolymerization under cyclic voltammetry conditions in aqueous solution. Anion of the biotin salt served as counterion for charge compensation of electro-oxidized polypyrrole. Electropolymerization was interrupted at the positive potential limit so, that anions could stay in the polymer. The avidin and albumin adsorption was studied by EQCM under flow injection analysis (FIA) conditions. First, consecutive injections of the avidin solution samples resulted in the negative frequency steps indicating that avidin irreversibly interacted with the biotin sites immobilized in the poly(pyrrole) film. The height of these steps decreased with the injection number as the biotin sites became gradually saturated. Then, adsorption of albumin on the saturated with avidin surface of the polymer film was examined. Morover, adsorption of avidin onto adsorbed layer of albumin was investigated. Results of the FIA experiments indicated that the avidin layer immobilized onto the polypyrrole-biotin film effectively repelled albumin.

10:35 Poster 20

Formation and properties of donor-acceptor dyads of Zn porphyrins and C_{60}-imidazole adduct in the Langmuir and Langmuir-Blodgett Films

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1. *Polish Academy of Sciences, Institute of Physical Chemistry, Kasprzaka 44/52, Warszawa 01-224, Poland* **2.** *University of Geneva, 24 quai Ernest-Ansermet, Geneva 1211, Switzerland* **3.** *Wichita State University, Department of Chemistry, 1845, Fairmount, Wichita, KS 67260-0051, United States* **4.** *Laboratoire de Pysicochimie Moléculaire - Université Bordeaux I, 351 Cours de la Libération, Talence 33405, France*

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In search for donor-acceptor dyads capable of photoinduced charge separation, being thus suitable for constructing organic photovoltaic elements, we examined self assembly of C_{60} adduct of imidazole via complex formation with Zn porphyrins in the Langmuir and Langmuir-Blodgett, LB, films. The Langmuir films of the C_{60}-imidazole adduct, C_{60}im, were prepared at the interfaces of air and aqueous subphase solution of water-soluble porphyrins, viz. Zn tetrakis (N-methylpiridyl)porphyrin cation, Zn(TMPyP), or Zn tetrakis (4-sulfonatophenyl)porphyrin anion, Zn(TPPS). In effect, relatively stable donor-acceptor dyads of C_{60}im-Zn(TMPyP) and C_{60}im-Zn(TPPS) were formed in the interfacial films. Comparison of the determined and calculated values of area per molecule, being dependent on the composition of the subphase solution, indicated that dyads were oriented with their porphyrin macrocycles in plane of the air-solution interface. Two control experiments indirectly confirmed that dyads were formed through axial coordination at the air-water interface. In the first experiment, spreading properties of the ligand-containing adduct floating on the surface of water or aqueous solution of Zn(TMPyP) were compared with those floating on solutions of free-base porphyrin, TMPyP. In the second one, spreading behavior of adducts containing imidazole ligand, C_{60}im, was compared with that of a blank adduct, viz. that containing a phenyl substitutent, C_{60}ph, floating on the surface of water or aqueous solution of Zn(TMPyP). Calculated by molecular modeling thickness of the Langmuir films was in accord with that determined by ellipsometry. The Langmuir films were transferred, by using the LB technique, onto different solid substrates for spectroscopic, microscopic, electroanalytical and photochemical characterization. It appeared that the Zn porphyrins were transferred together with the adduct onto solid substrates. Orientation of the dyad molecules in the LB films was detemined by IR spectroscopy. Molecularly modeled hexagonal packing and thickness of the LB films was in accord with that measured by the STM imaging and determined by ellipsometry, respectively. The electrochemical redox states of the dyads were established by performing simultaneous cyclic voltammetry and piezoelectric microgravimetry measurements on the LB films deposited on the Au-quartz electrodes. Time-resolved emission studies of the fullerene-(Zn porphyrin) LB films revealed efficient quenching of the singlet-excited Zn porphyrin. Based on the free-energy calculations and dyad orientation in the film, this quenching was attributed to vectorial electron transfer within the dyad.

10:35 Poster 21

Composition, structure, surface topography, and electrochemical properties of electrophoretically deposited fullerene films

Wlodzimierz Kutner[1], Piotr Pięta[1], Robert Nowakowski[1], Janusz W. Sobczak[1], Zbigniew Kaszkur[1], Amy Lea McCarty[2], Francis D'Souza[2]

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Fullerene films of controlled surface topography were prepared by electrophoretic deposition from toluene-ethanol mixed solvent solutions. The AFM imaging of the films revealed that the size of the C_{60} grains in films could be readily controlled both with the time of C_{60} aggregation in bulk solution before the deposition and strength of the applied dc electric field. The film deposition was monitored by piezoelectric microgravimetry with the use of an electrochemical quartz crystal microbalance. The mass of the C_{60} film increased exponentially with the time of deposition. The corresponding decrease of the deposition rate with time was presumably due either to the growth of larger C_{60} aggregates in solution of lower mobility or blocking effect of the electrode surface by insulating C_{60} deposits. In the accessible potential window of 0.1 M $(TBA)PF_6$, in acetonitrile, cyclic voltammetry curves for the C_{60} films featured four cathodic peaks corresponding to four one-electron reductions. Simultaneously recorded multi-scan curves of current, resonant frequency change, and dynamic resistance change vs. potential for a potential range covering the first two electro-reductions (that is the range in which the film was relatively stable with respect to dissolution) indicated pronounced transformations in the film caused by the solvent-assisted dynamic equilibria of ions of the supporting electrolyte. The related to C_{60} charge in films piezoelectric microgravimetry, XPS, and XRD analyses indicated reversible ingress of both TBA^+ counter- and $PF_6^{\square}$ co-ion into the C_{60}^- film.

Kazimierz Dolny - sightseeing

walking tour, if weather allows for it

Monday morning, 7 November, 11:00

Lunch

Monday afternoon, 7 November, 13:30

SESSION 2

Chairpersons: Andrzej Lewenstam, Bengt Danielsson

Monday afternoon, 7 November, 15:00

15:00 Tutorial lecture

Designed affinity surfaces for biomolecule immobilisation and biosensor construction

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Nanoscale design and functionalisation of transducer surfaces is vital for the effective and rapid assembly of biosensors devices. Substrates can be initially functionalised in a variety of ways; screen printed carbon electrodes have been aminated of via electrochemical reduction of nitrobenzene diazonium, whilst silica or platinum have been aminated via treatment with aminosilanes such as APTES; finally gold/silver are most easily modified but thiol-terminated reagents which chemisorb to the metal surface. An alternative strategy with conducting surfaces is the electropolymerisation of conducting polymers such as polyaniline and polypyrrole, which also provide surface amino functions for subsequent modification.

Once the base layer is in place then various affinity strategies exist for rapid and gentle attachment of the sensing biomolecule, usually an enzyme or antibody. Non- specific affinity strategies have been used to capture enzymes and antibodies. Acetylcholine esterase was immobilised via multipoint electrostatic interaction to multilayers of the polyanion PEI, to construct an organophosphate pesticide sensor. Antibodies to various analytes have been tethered in the same way to polypyrrole and polyaniline bases layers on Au and carbon using multiple alternate layers of the polyanion PDDM and polycation PSS.

Specific affinity interactions have also been used for biomolecule immobilisation. Attachment of a biotin group to surface amines on polyaniline subsequently permits tethering of biotinylated antibody via avidin as a multivalent crosslinker. The same strategy has also been used to attach biotinylated antibodies to transducer surfaces supporting a mixed self assembled monolayer, which comprises the alkanethiol MHDA and intercalated affinity lipid, DPPE-caproyl-biotin. A similar approach can be used to immobilise recombinant sensor molecules, e.g. Fab, or Scfv, which bear a terminal His_6 tag, to the affinity lipid DPPE-Ni^{2+}-NTA. Such mixed self assembled monolayer incidentally also function well as a 'non-specific' His_6' tagged protein sensor.

Finally, approaches to characterising the assembly of such nanostructured surfaces and biosensors will be described. These include quartz crystal microbalance (QCM) to observe the kinetics of sensor assembly and analyte recognition, AFM to delineate surface topology, radiolabelling components for quantification, and amperometric and impedimetric interrogation of the sensor at various stages of assembly.

15:40 Keynote lecture

Membrane proteins in tethered bilayer lipid membranes

Renate L. Naumann, Wolfgang Knoll

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Membrane Proteins in Tethered Bilayer Lipid Membranes

Renate Naumann and Wolfgang Knoll

The tethered Bilayer Lipid Membrane, tBLM, has been introduced as a model system of the biological membrane. Membrane proteins are shown to incorporate in a functionally active form into tBLMs in two different formats:

Thiolipid-based tBLMs have been used to incorporate porines, ion carriers and channels such as hemolysin, valinomycin, melittin, gramicidin [1], MaxiK, the M2 channel of the AChR. The kinetics of ion transport has been analysed using impedance measurements. The spectra are simulated using the computer program Spice designed to analyze bioelectrochemical processes.

Larger complex proteins such as the cytochrome c oxidase from *Rh. sphaeroidis*, CcO, are incorporated in the so-called **protein-tethered Bilayer Lipid Membrane (ptBLM)** developed in our laboratories. Recombinant CcO is immobilized gold surface to a nickel chelating nitrilo-triacetic acid (NTA) surface. The CcO monolayer is reconstituted into the lipid by in-situ dialysis.

Direct electron exchange between the gold electrode and the CuA redox center of the CcO is then investigated by electrochemical methods combined in a spectroelectrochemical cell with surface enhanced resonance raman spectroscopy (SERRS) .

The combined information is discussed in terms of current models of the mechanism of the CcO.

16:00 Short communication

Rectification behavior of molecular junctions incorporating peptides

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Self-assembled monolayers of alpha-helical peptide on gold surface were employed as model systems for the investigations of mediated electron transfer. The measurements of electron transfer efficiency through single helical peptide molecules were performed using scanning tunneling spectroscopy (STS). The molecules were trapped between the gold tip and the substrate. The electrical contact between the molecule and the gold probe was achieved by the use of peptides containing thiol groups present at each end of the helix. The electron transfer behavior of the peptides was examined as a function of tip-substrate distance at fixed bias voltage and as a function of bias voltage at a fixed distance between the tip and the substrate. The data obtained from these experiments indicated that there is a directional dependence of electron transmission through peptide. It is suggested that the observed electron transfer behavior is connected with the electric field generated by the molecular dipole of the helix.

16:15 Short communication

Assessment of electroanalytical redox scaffolds for use as a biosensor

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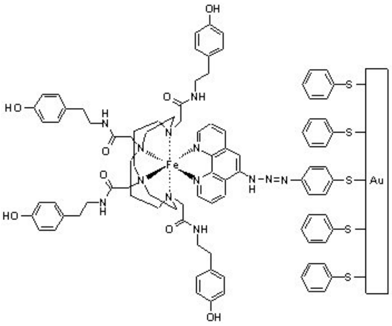
Traditional biosensors rely on redox enzymes to turnover the analyte. In this case, it is the by-products of these reactions, or mediator substituents, rather than the analyte itself that is detected. The thrust behind this work is to design a biosensor that allows direct detection of the analyte, utilising a recognition element without turning over the analyte.

The sensor is based upon a redox scaffold, which is functionalised with an analyte recognition site. The analyte binding event is detected by a shift in the redox potential of the scaffold. The scaffold is bound to an electrode to allow direct electron transfer between it and the redox centre. This concept has been tested using a tyramine antibody as the analyte and tyramine as the analyte recognition site.

1,4,7,10-tetraazacyclotetradecane (cyclam) is presented as the scaffold, as it forms kinetically and thermodynamically stable complexes with a wide variety of transition metals and is readily functionalised. Introduction of pendant arms on the macrocyclic scaffold lead to a significant (anodic) shift in redox potential.



A model redox-recognition scaffold is proposed (shown above), and the results presented, to test cyclam as a core biosensor reagent. The scaffold is immobilised via aminophenanthroline through diazo groups on to a mixed SAM on a gold electrode, thereby permitting direct electron transfer from scaffold to electrode.

Coffee break/Poster session

Monday afternoon, 7 November, 16:30

SESSION 3

Chairpersons: Wolfgang Schuhmann, Konrad Szacilowski
Monday afternoon, 7 November, 16:55

16:55 Tutorial lecture

Direct bioelectrocatalysis by the enzymes. Towards hydrogen sensor and hydrogen-oxygen biofuel cell

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Bioelectrocatalysis is a phenomenon of acceleration of electrode reactions with biological catalysts. Most often the immobilized enzymes are used as the latter. The phenomenon of 'direct bioelectrocatalysis' presumes a possibility for direct (mediatorless) electron exchange between the electrode and the enzyme active site. In this case the electrode reaction substitutes directly one of the coupled redox reactions catalyzed by the enzyme.

The discovery of the direct bioelectrocatalysis is a proud of Russian electrochemical school. In late 70-s of the last century the enzyme electrodes active in reduction of oxygen and hydrogen peroxide made on the basis of immobilized laccase and peroxidase, correspondingly, were reported. In the beginning of 80-s a hydrogen enzyme electrode able to achieve hydrogen equilibrium potential was elaborated.

Fundamental aspects of direct bioelectrocatalysis include: (i) the investigation of the enzymes redox centers and electron transport chains, and (ii) a possibility to access enzyme catalysis, in particular to carry out redox switching of enzyme activity.

Besides fundamental aspects, the direct bioelectrocatalysis is extremely attractive concerning its applications. Among them are: (i) biosensors, (ii) biofuel cells, and (iii) systems for specific electrosynthesis. Except for the last one, the applications are rapidly developed. I will mention the biosensors for hydrogen peroxide based on peroxidase, and bioelectrocatalysis by hydrogenase and laccase used as for biofuel cells, as for analytical purposes.

Special attention will be devoted to hydrogen-oxygen fuel cells. Besides the majority of efforts are currently put on the development of platinum based fuel electrodes, there are crucial problems with platinum, which seriously limit the use of such systems in future. A valuable alternative to platinum based electrocatalysis is the bioelectrocatalysis. The advantages of the enzymes based fuel electrodes in hydrogen-oxygen fuel cells (including systems for utilization of organic wastes) will be discussed.

In addition to fuel cell applications, hydrogen enzyme electrodes can be used as hydrogen sensors, where the use of platinum is impossible due to high contamination with sulphide and other platinum poisons. A possibility for hydrogen sensing directly from microbial medium will be shown.

17:35 Keynote lecture

Redox-flexible ferrocene mediated oxidase based biosensors. Study of competitive reactions

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The diversity and complexity of food matrix as well as the necessity for simple, sensitive and selective sensors encouraged us to develop the concept of "redox-flexible" modified carbon electrode containing several enzymes and mediators that can be efficiently used at different working potentials.

For amperometric biosensors the choice of the working potential is a real advantage as it permits to escape various interferences which are related manly to the complexity of the sample. Enabling the biosensors to work either on anodic or cathodic modes associated with the expansion of the working potential window increase the flexibility of the use of enzymatic biosensors in real samples.

In this lecture we will show how for oxidase enzymes based bio-assays and biosensors (glucose oxidase, lactate oxidase, NADH oxidase, cholesterol oxidase) the simultaneous association of horseradish peroxidase with a mediator, competitor or not with the natural co-substrate (O_2) of the enzyme permits to generate two different pathways for the analyte detection: one operating in anodic mode and the other in cathodic mode. The role of competitive reactions resulting from the association of different enzymes with the mediator will be also discuss.

The studies presented here were performed using disposable screen printed electrochemical cells with the carbon working electrode modified by the redox mediator and enzymes.

17:55 Keynote lecture

Liquid photopolimerized compositions as immobilization matrix for biosensors

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Series of liquid photopolimerized compositions (LPhPC) based on oligouretanemetacrylate (OUM-1000T and OUM-2000T) and oligocarbonatemetacrylate (OCM-2), monomethacrylic ether of ethylen glycol and vinylpirrolidone (VP) were tested. It was shown that the optimal LPhPC contained VP (as basic hydrophylic matrix), OCM-2 (crosslinked agent) and OUM-2000T (crosslinked and increasing adsorption of polymer). The blend contained 3% of enzyme. ISFETs based biosensors for glucose or urea had following charachteristics: response was linear in the the concentration of 0,1-10 mM, 0,05-20 mM, slope angle of curve - 30 mV/pC, 38 mV/pC, and response time - 10-15, 5-10 min, correspondingly. Km for immobilised urease and β-glucose oxidase (GOD) achieved values of 0,85 and 3,1 mM, respectively. The residual activity of enzymes in LPhPC was studied. Under conditions of the immobilization at 20 ºC GOD residual activity is about 35 % from an initial level, horseredish peroxidase and urease - 42% and 20%, respectively. In case of GOD immobilization at -50ºC its residual activity reaches almost 50% from initial level. It was investigated the influence of different sources of UV-radiation and different substances (including specific and non-specific substrates) on stability of the enzymes in the LPhPC and in the prepared membrane at storage. Dynamic of changes of enzyme activity at the process of photoimmobilization was characterized, and also the requirements for providing of its maximal storage was selected. So, it was proposed the optimal conditions for preparation of LPhPC to meet biosensorics demands in respect of non-active for biological substances, permeability for analytes, defined hydrophobic-hydrophilic balance, sufficient level of adhesion to transducer surfaces, simplicity of immobilization process and homogeneity of formed membrane.The proposed LPhPC may be preliminary prepared since enzymes did not lost or lost a few bit their activity during 2 month and it may be used for combination of two processes: manufacturing transducers and simultaneous biological membrane preparation by traditional approach of modern electronics - photolithography.

18:15 Short communication

Thermoresponsive polymeric hydrogels anchored at gold surfaces and used as matrix for enzymes

Marianna Gniadek, Marcin Karbarz, Zbigniew Stojek

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We have focused our attention on polymeric hydrogels that are cross-linked polymer networks filled with water. The content of water in hydrogels may be higher than 95%. Some of these polymers can exist in two different states: swollen and collapsed (shrunken). The transition from one state to another is called the volume phase transition. This transition is a response to the changes in such conditions as temperature [1] and pH [2]. When a hydrogel in a swollen state undergoes the volume phase transition, water is removed from the polymeric network.

The unique structure and environmental sensitivity of the polymeric gels make them useful in various applications including, drug delivery systems, separation techniques, and construction of sensors. In the development of such applications the knowledge of the internal structure and the mobility of the molecules and ions in these materials and the influence of the environmental conditions on these properties is very useful. The electroanalytical methods offer a fast, inexpensive and accurate approach to such examinations [3].

The discontinuous change in the volume of the gels examined by us occurs when temperature exceeds 34 ^{0}C. The first step was to anchor a thin film of NIPA {cross-linked poly(N-isopropylacrylamide)} hydrogel to the surface of a gold electrode. To achieve this, the surface of the electrode, before the polymerization process, was treated with dimetylvinyletoxysilane. The schematic structure of the NIPA polymer linked with the electrode through the dimetylvinyletoxysilane is shown in the figure. Then we attempted to examine the volume phase transition of the gels in the attached state and to find out whether the adhesion of the layer to gold is strong enough not to permit the peeling of the polymer layer during the shrinking process. For these examinations we have employed various electroanalytical and optical methods. The shrinking behaviour the NIPA hydrogel attached to the gold surface was compared with that of a free, grown in a capillary, gel sample. The anchoring of thin layers of NIPA hydrogel was apparently successful.

The electrode with the anchored gel appeared to be a good matrix for immobilization of the enzymes. Some tests with glucose oxydase and laccase have been made. The enzymes introduced to the matrix during its polymerization growth did not loose their activity. This was confirmed electrochemically and spectrometrically.

[1] X. Z. Zhang , J. T. Zhang, R. X. Zhuo, C. C. Chu,. *J. Chem. Phys.,* **43** (2002) 4823.
[2] K. Ogawa, A. Nakayama, E. Kokufuta, *Langmuir,***19** (2003) 3178.
[3] W. Hyk, M. Karbarz, Z. Stojek, M. Ciszkowska, *J. Phys. Chem. B,***108** (2004) 846.

Dinner

Monday evening, 7 November, 18:30

SESSION 4

Chairpersons: Elzbieta Malinowska, Martial Billon

Monday evening, 7 November, 20:00

20:00 Tutorial lecture

Bioanalytical studies based on lectin-carbohydrate interactions measured by the ellipsometry and SPR techniques

Jana Masárová[2], Jenny Carlsson[1], Fredrik Winquist[1], Estera S. Dey[2], Bengt Danielsson[2]

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Biosensors use a combination of biological receptor compounds and the physical or physical-chemical transducer directing real-time monitoring of specific biological events. Surface plasmon resonance (SPR) technique is a powerful optical biosensing technique for non-label affinity interaction analysis. Lectins as biological receptor molecules and Biacore 3000 as optical transducer were employed for bacteria differentiation. The lectins were covalently immobilized on Biacore CM5 chips and the solution containing only endotoxin (lipopolysaccharide) or whole bacterial cells was flowed over the surface. The obtained lectin binding patterns well correlated with theoretical pattern derived from carbohydrate structure of endotoxin. The experiment with whole bacteria showed possibility to distinguish bacteria with cross-reactivity in immunoassay (Citrobacter freundii and Escherichia coli O157) by using of the lectin panel. The expression of carbohydrate structures on the outer side of the bacteria was required for increase the sensitivity of measurement.

In an earlier study [*Anal. Chim. Acta*, **459** (2002) 25] a similar set of lectins was used to discriminate between healthy and infected serum samples. Fine tuning of this technique is under investigation. This study was also carried out with a Biacore instrument. Faster measurements can be made with scanning or imaging ellipsometers. Sera from human, pig, sheep and guinea pig were applied to a panel of eight different lectins immobilized on a gold wafer. The 2-D matrices obtained by scanning ellipsometry were evaluated with image analysis and multivariate data analysis. There was a clear difference in protein binding pattern between the different species and dendrograms indicated that human and pig sera are the most related of the four different sera [*Anal. Chim. Acta*, **530** (2005) 167]. Similarly, simple micro contact-printed gold-wafers were used to make a lectin panel for investigation and discrimination of different meat juices from fresh meat of cattle, chicken, pig, cod, turkey and lamb. Using a simple model based on an artificial neuronal net, it was also possible to classify meat juices from the mammals investigated [*Anal. Chim. Acta*, **547** (2005) 229].

20:40 Keynote lecture

Electron transfer applications of nanostructured supramolecules

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Utilization of self-assembly methods to build exotic nanostructured supramolecules of well-defined structure and topology, and to be capable of undergoing specific functions such as electron transfer, ion or molecule recognition, biomimicry, catalysis, sensing, and self-replication is one of the main themes of modern chemistry. Several fascinating supramolecular structures of few nanometers size have been constructed using a variety of self-assembly approaches, and some of them have been shown to perform one or more above mentioned functions. Our interest in this area of research has been to self-assemble metallotetrapyrrole macrocycles to electron acceptors like quinone and/or fullerene in solution and at surfaces for probing their electron transfer, catalytic and sensor applications. The present tutorial talk will discuss some of the recently developed self-assembly methods in our laboratory for porphyrin-quinone and porphyrin-fullerene conjugate formation. Electron transfer in solution and at surfaces originating from these donor-acceptor conjugates will be discussed. Chemosensor applications of these novel supramolecules especially to detect neutral analytes will also be discussed.

Tuesday, 8 November

Breakfast

Tuesday morning, 8 November, 7:30

Bus excursion to Kozlowka (Museum of Communism)

Tuesday morning, 8 November, 8:30

Lunch

Tuesday afternoon, 8 November, 15:00

SESSION 5

Chairpersons: Włodzimierz Kutner, Alexander Kuhn

Tuesday afternoon, 8 November, 16:30

16:30 Tutorial lecture

Probing organization and communication at layered interfaces

Maciej Mazur[1], Monika Dominska[2], Paweł G. Krysiński[1], Gary J. Blanchard[2]

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Many present-day technologies, such as catalysis, chemical sensing and nanoelectronics, rely on phenomena that proceed at interfaces. Because of the limited structural control we can exert over solid surfaces, an attractive strategy for tailoring interfaces to specific purposes is growing monolayers or multilayers onto them. Such adlayers can be prepared by several methods, with Langmuir-Blodgett and self-assembly being the most useful and widely used approaches for interface structural control. The chemical modification of interfaces requires the development and use of measurement techniques that are capable of probing the interfaces once formed. Both electrochemistry and spectroscopy are useful for this task, depending on the interface used. For both electrochemical and spectroscopic measurements, the incorporation of probe molecules into the modified interface enhances the sensitivity and specificity of the information gained. Fluorescent spectroscopic probes have proven especially useful because they can be detected with high sensitivity and in some cases the details of their emission profile are reflective of their immediate environment. We use pyrene as an optical and electrochemical probe for this work because of its ability to sense the "polarity" of its microenvironment through the intensity ratio of two emission bands. These two bands respond differently to the dielectric properties of the chromophore immediate environment. For substituted pyrene, the emission band ratio environment dependence is somewhat less sensitive than for pyrene because of the reduction in symmetry, but if chosen properly, substituted pyrene can be used as a probe of interface local environment. We report on organization and intermolecular communication within several interfacial systems as a function of interface composition and for immersion of the interface in selected solvents. The co-deposition of selected molecules with pyrene derivatives has a measurable effect on the pyrene emission spectrum, and the details of this effect depend on the identity of the surface modifier. We gauge the sensitivity of vibronic band intensities in the emission spectrum of pyrene by co-immobilizing hydrophobic molecules, demonstrate energy transfer in the system of two spectrally overlapped chromophores, and demonstrate that pyrene can communicate with other electrochemically active species within the interface. The average distance between molecules in these interfaces is nanometers, close enough for efficient communication. This fact is important from fundamental and technological standpoints, as in the construction of sensors and the design of nanoelectronics, and in understanding chemical deposition at heterogeneous interfaces.

17:10 Tutorial lecture

New methods of immobilization of oxidases at electrodes

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Retaining enzymes in functionally-active forms on the electrode surface is a challenging and difficult task. Ideally, the immobilization of protein should be performed under conditions that provide membrane like environment in which all the normal interactions of the proteins are preserved. Providing electronic contact of the protein molecules with the conducting substrate by means of a biocompatible medium is even more difficult. In the present talk several ways of contacting oxidases with electrodes will be discussed.Three step sequential self-assembly procedure was applied to prepare gold electrodes functionalized in a stable and controlled way with a β-cyclodextrin (β-CD). When thiolated cyclodextrins are used as active sites in the organothiol monolayers modifying electrodes, the electron transfer from solution species to the electrode is facilitated in the presence of appropriate mediators in the cyclodextrin cavity. Methylene blue in the cavities provided electrical contact between the electrode and solution resident enzyme - laccase catalyzing reduction of oxygen to water. Covalent binding of laccase to thiol monolayers using 11-mercaptoundecanoic acid as the organic junction was found to provide direct electrical contact with the enzyme together with its stable immobilization at the electrode. Direct electrochemistry of the enzyme can be observed and its catalytic activity demonstrated. Immobilization in thin films of lyotropic liquid crystal - lipidic matrix with cubic symmetry is discussed as a way of immobilizing enzymes by means of hydrophobic-hydrophilic interactions with lipid molecules. The electrochemical behaviour and method of immobilizing the fungal laccases in diamond type monoolein cubic phase will be discussed. The cubic phase prevents the enzymes from denaturation on the electrode surface, and provides contact of enzyme with the electrode. Thin layer of the cubic phase embedding glucose and pyranose oxidase activated glucose oxidation. The potentials and ratio of catalytic to diffusion currents are modified by choosing the appropriate electroactive probes as mediators. Ferrocenecarboxylic acid and $Ru(NH_3)_6^{2+}$ provided good contact between the electrode and the enzyme. The system based on $Ru(NH_3)_6^{2+}$ was found most suitable in terms of catalytic efficiency, low potential.

Acknowledgement: This work was financially supported by Ministry of Scientific Research and Information Technology - Project No. PBZ 18-KBN-098/T09/2003.

17:50 Keynote lecture

Unusual photoelectrochemical behaviour of surface-modified nanocrystalline semiconductors

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Surface-modified nanocrystalline semiconductors have found numerous applications as (photo)catalysts, chemosensors and biomimetic light-harvesting antennae. Systems containing molecular switches attached to the surface of titania were recently described. Up to date there are no detailed reports on mechanisms and applications of photocurrent switching phenomena in cyanoferrate-modified titanium dioxide and cadmium sulfide. The increasing interest in molecular electronics stimulated the research on switching properties of some semiconductor composite materials.

This presentation concerns the photoelectrochemical properties of multicomponent photoelectrodes based on nanocrystalline titanium dioxide and cadmium sulfide modified with hexacyanoferrate complexes. Photocurrent responses were recorded as functions of applied potential and photon energy. The photocurrent can be switched between positive and negative values as a result of electron and/or photon energy changes. This new effect called PEPS (PhotoElectrochemical Photocurrent Switching) opens a possibility of new light-harvesting antennae, chemical switches and logic gates construction. Boolean logic analysis and a tentative mechanism of the device are presented. Complex switching patterns allow for simulation not only simple logic gates but also more complex logic systems.

18:10 Short communication

Divalent cations effects on the formation and structure of solid supported lipid films

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Solid supported lipid films have proved very useful both as biomembrane mimetic analogs and as a basis for electrode modifications in the construction of sensor devices. The films are prepared in quite different manner, including liposome spreading, self-assembly, Langmuir-Blodgett (LB), or cast film technique. An alternative method of formation is achieved through the thinning of wetting lipid films on solid surfaces, submersed in electrolyte solution. The impact of some divalent cations on the structure of a model membrane system has been investigated with several types of supported lipid film, comprising wetting lipid films. Changes in the films capacitance and resistance and opening of channel-like defects in supported lipid films have been reported. This work represents further investigations of the effects caused by the divalent cations on the formation and structure of wetting lipid films. The impedimetric and voltammetric results suggest significant influence of Mn^{2+}, Mg^{2+}, Ca^{2+} on the packing order and strengthening of the lipid structure of the film.

18:25 Short communication

Detection of clinically relevant point mutations by a novel piezoelectric biosensor

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TP53 gene is an important early diagnostic cancer marker since it mutated in most type of human cancers and *TP53* mutations have prognostic importance and sometimes are a significant factor in determining the response of tumours to therapy. A DNA-based piezoelectric biosensor has been developed for the detection of *TP53* mutation using the new commercially available instrument QCMagic (Elbatech-Italy). This instrument with 9.5 MHz quartz crystals has been applied to the detection of *TP53* point mutations (codon 248) in PCR amplified samples. The affinity reaction is based hybridisation reactions between the immobilised probe and a short sequence (26 mer) complementary, non-complementary and one-point mutation DNA were then investigated. The biosensor is first developed using synthetic oligonucleotides, studied in terms of the main analytical parameters (sensitivity, specificity, reproducibility, etc.) and then applied to the analysis of real samples consisting in amplified DNA extracted from "normal" wild type cell line (Jurkat) containing the fully complementary sequence and DNA extracted from cell line (Molt 4) containing the mismatch sequence.

Dinner

Tuesday evening, 8 November, 18:40

SESSION 6

Chairpersons: Krzysztof Winkler, Arkady Karyakin
Tuesday evening, 8 November, 20:00

20:00 Tutorial lecture

Novel solution-free reference electrodes for potentiometric applications

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In recent years a considerable progress in construction of potentiometric sensors has been achieved in the area of miniaturization, disposability and internal solution free construction. However, for the reference electrodes, still classical constructions based on Ag/AgCl or Hg/Hg_2Cl_2 dominate. The profound stability of these highly reliable electrodes is accompanied by a serious disadvantage: presence of a liquid solution phase connected with need of refilling / maintenance and restriction of vertical working position. Moreover, these arrangements are often difficult to suit with modern miniaturized sensing devices and their production methods.

Among requirements for reliable reference electrodes, independence of recorded potentials of changes of sample activity and composition is of crucial importance from the point of view of applications in potentiometric ion sensing cells. Generally, liquid-free reference electrodes need solid conducting materials and either a reversible redox system or a membrane non-selective to ions, to assure a constant potential independent of changes in the sample medium. Recent achievements in this field will be reviewed.

The results of our recent work, connected with applications of conducting polymers (CP) for reference electrodes construction will be also presented. In this work two different arrangements have been tested: solely based on CP and those where the CP phase is covered with a poly(vinyl chloride) based outer membrane. The former system has been tailored to suppress or compensate both cation- and anion-exchange properties, by appropriate using of mobile and immobilized dopants.

The best results, profound stability of potentials and promising electrical properties have been obtained for poly(3,4-ethylenedioxytiophene) or poly(pyrrole) doped by poly(4-styrenesulphonate) anions covered by a poly(vinyl chloride) based membrane, containing both anion- and cation-exchangers as well as solid potassium chloride and silver chloride with metallic silver traces.

20:40 Keynote lecture

Multiscale tailored electrode surfaces in bioelectrocatalysis

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In the last few years we have developed and used approaches that allow us to tailor the physico-chemical properties of modified electrodes at several scales. The different scales of organisation include: **(a)** the molecular scale for the engineering and the surface self-assembly of a redox mediator [1,2] **(b)** the supramolecular scale for the surface assembly of mediator, coenzyme and enzyme using non-covalent interactions [3,4] **(c)** the nanometer to submicrometer scale for the highly organized meso- and macroporous electrode surfaces [5,6] **(d)** the micrometer scale for the functionalised microelectrodes obtained by using ingredients (a)-(c). In this way, the overall bioelectrocatalytic current can be easily amplified by more than one order of magnitude, as compared to a flat electrode surface. This allows to increase significantly the detection limit and sensitivity of such a device when used as a biosensor, or the power output in the case of a biofuel cell.

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Wednesday, 9 November

Breakfast

Wednesday morning, 9 November, 8:00

SESSION 7

Chairpersons: Frank Marken, Marcin Opallo
Wednesday morning, 9 November, 9:00

9:00 Tutorial lecture

New strategies in the electroctalytic reduction of oxygen for fuel and biofuel cells

Paweł J. Kulesza, Krzysztof Miecznikowski, Katarzyna Karnicka, Andrzej Ernst, Malgorzata Chojak, Beata Baranowska, Magdalena Skunik, Barbara Starobrzynska

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Platinum has been established as the most powerful electrocatalyst for the 4-electron reduction of oxygen to water in acid medium in a conventional fuel cell. The reaction becomes less efficient and its efficiency suffers from the formation of the hydrogen peroxide intermediate at lower Pt loadings or at higher current densities. Recent progress in the area concerns fabrication of electrocatalysts in a form of carbon-supported platinum nanoparticles assembled within organized multilayers or inks deposited on a proton-conducting membrane. Important issues include the stabilization of Pt nanoparticles (to prevent their agglomeration causing decrease of the active surface area), the enhancement of their reactivity (e.g. by modification of their surfaces or by formation of novel bimetallic systems), the interfacial protection of nanostructured Pt from poisoning (e.g. by the development of methanol or CO tolerant systems using Ru-Se nanoparticles), and the activation of metal nanoparticles through the immobilization within reactive matrices (bifunctional mechanism). The above studies are paralleled by more or less successful attempts to develop the noble metal free electrocatalysts (e.g. metal phtalocyanines, porphyrins and their including pyrolyzed structures) of potential utility to fuel cells operating in acid media.

Biofuel cells can be viewed as analogues of conventional fuel cells in which biocatalysts (enzymes), biofules, and neutral or slightly acidic electrolytes are used. The interest in biofuel cells has been raised by biomedical applications and environmental concerns. Since most of conventional electrocatalysts for oxygen reduction are largely useless in neutral media, the present research centers on development of the methods of immobilization of enzymes (highly specific but slow electron-transfer biocatalysts) within redox mediators at electrode surfaces. Among present limitations is not only the long-term stability and reactivity of enzymes but also the durability and ability of polymeric redox mediators to propagate charge (electron transfers, displacement of ions) to the redox centers. When referring to the theory and methodology of electrocatalysis at polymer modified electrodes, care has to exercised to produce highly conducting (electronically, ionically) three-dimensional redox polymer films containing possibly the highest concentration of catalytic (enzymatic) centers. Recent examples of redox polymers, the choice of enzymes, and the possibility of use of carbon nanostructures will be discussed. The concept of bifunctional electrocatalysis with use of both biological and inorganic redox centers will be addressed too.

9:40 Keynote lecture

Determination of redox properties of semiconducting materials

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Chemical and electrochemical surface processes attract a growing attention due to their possible practical applications. In particular, semiconducting materials and their modifications are used as catalysts and photocatalysts, electrodes, (bio)sensors *etc*. One of the crucial feature of an appropriate semiconductor suitable for a given purpose is the position of valence and conduction band edges at the energy (or potential) scale. For instance, the redox properties of photogenerated holes and electrons determine the activity of a photocatalyst. Therefore determination of redox potentials of semiconducting materials is very important. This knowledge helps to select the proper material and to elucidate mechanisms of processes taking place at the surface.

Although the energy bandgap can be relatively easily estimated from reflectance spectra of semiconductors, the measurement of band edge potentials is more difficult. One of the methods is based on capacity measurements. From the Mott-Schottky plot (the space charge capacity *vs.* electrode potential) it is possible to determine the so called flatband potential. The capacitance method is useful for semiconductor electrodes but not for suspensions. A number of other methods based on spectroscopic determinations, modulation spectroscopy, photocurrent, and photovoltage measurements have also been developed. In this presentation selected methods will be presented. Simple photoelectrochemical and spectroelectrochemical methods suitable for flatband potential measurements for semiconducting powders and transparent films will be discussed in detail.

10:00 Short communication

Polyoxometallate-modified conducting polymer linked Pt nanoparticles as bifunctional electrocatalysts for bromate reduction

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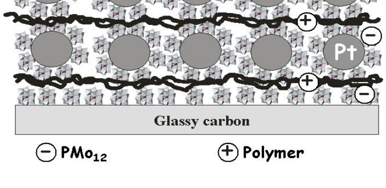
Recently, there has been tremendous interest in the fabrication and characterization of monolayer and multilayer organized assemblies at solid surfaces that produce functionalized interfaces with well-defined composition, structure, thickness and reactivity. Attractiveness of the self-assembly technique includes the possibility of controlled and sequential formation of any number of layers of different composition (e.g. of oppositely charged polyelectrolytes, ultra-thin films of conjugated polymers) on a large variety of substrates. Also the possibility of stabilizing of nanoparticles and their organization into two-dimensional arrays and three-dimensional networks have prompted the increased interest due to their potential applications in many areas including sensors, catalysis and electrocatalysis.

An important possibility arises from the adsorption of inorganic monolayers. Among rigid inorganic templates, *polyoxometallates* are particularly attractive because of their ability to adsorb irreversibly on solid surfaces [1,2] as well as on metal nanoparticles (e.g. platinum and their alloys) [3].

In the present study, we demonstrate that the electrodes modified with PMo$_{12}$-covered platinum nanoparticles (inter-connected via polypyrrole layers as demonstrated in Scheme) exhibit remarkable electrocatalytic effect on the reduction of bromate, a highly toxic substance (carcinogen), which may be present in drinking water when ozonation is applied in the treatment of bromide-containing water. An important issue is the bifunctional electrocatalytic effect originating from the ability of both Pt and PMo$_{12}$ to induce reduction of bromate. What is analytically important, PMo$_{12}$ (that is chemisorbed on Pt) inhibits platinum reactivity towards proton discharge and decrease background currents.



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10:15 Short communication

Nanostructure film modified electrodes for the preparation of DNA biosensors

Adriana Ferancova[1], Ludmila Heilerova[2], Jiří Zima[3], Jiří Barek[3], Jan Labuda[1]

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DNA modified electrodes have found a wide popularity among sensors for the biochemically related analysis. A variety of commertial electrode substrates was used to transduce the signal. In recent years, different nanostructured materials have became the subject of intensive investigations and applications because of their unique mechanical, electrical, optical and thermal properties. Nanoobjects such as nanoparticles of metals, semiconductors, oxides and nanostructures such as nanotubes and nanowires could significantly improve the sensitivity of electrochemical sensors and biosensors.

Nanostructured films of multi-wall carbon nanotubes, hydroxyapatite and montmorillonite were deposited at the surface of a working electrode of the commercial carbon paste-based screen-printed assembly (SPE) and utilized for the surface modification with the double-stranded DNA using layer-to-layer coverage and mixed (composite) coverage. The surface morphology of the sensors was investigated using scanning electron microscopy. The DNA redox marker [Co(phen)$_3$]$^{3+}$ and ferrocyanide ion were used for the investigation of basic electrochemical properties of the sensors using differential pulse voltammetry and cyclic voltammetry. The modified electrodes were characterized also by electrochemical impedance measurements.

The DNA-based biosensors showed good repeability of the signal as well as necessary stability within several days. A relative enhancement of the DNA signals at the SPE electrodes with nanostructured films comparing to the simple DNA modified SPE, which is neccessary for the detection of DNA binding and damage interactions, was found. DNA biosensors based on carbon nanotubes were also applied to the detection of damage to DNA.

Acknowledgement: This work was supported by the Grant Agency APVT (No. 27-010304 and No. 20-015904) and by the Grant Agency of the Czech Republic (No. 203/03/0182).

10:30 Short communication

Evaluation of TiN thin film electrodes for electroanalytical purposes using scanning electrochemical microscopy

Carolina Nunes Kirchner[1], Christian Radehaus[2], Gunther Wittstock[1]

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Scanning electrochemical microscope (SECM) can be used to extract first-order rate constants from approach curves in the feedback mode. For the analysis of more complex reaction mechanisms, this first-order rate constants can be related to different rate laws. In this example the conductivity of TiN thin film of different thickness was analysed. The experiment consisted in vertical approach of an amperometric ultramicroelectrode (UME) of controlled size and overall geometry to the sample surface (TiN film) while a reversible redox couple (ferrocene methanol / ferrocenium methanol) undergoes a diffusion-controlled electron transfer (et) reaction at the UME. The sample was biased at a potential so that it re-reduces the species being produced at the UME closing the reaction cycle. The UME current depends on the kinetics at the sample. At large tip-substrate distance the feedback is controlled by diffusion and as it approaches the surface is controlled by the kinetics of reactions at the substrate interface. The normalised current-distance curves were fit to the theoretical model in order to find the standard rate constant (k^0) and then conductivity.

Coffee break/Poster session

Wednesday morning, 9 November, 10:45

SESSION 8

Chairpersons: Renate L. Naumann, Krzysztof Maksymiuk

Wednesday morning, 9 November, 11:10

11:10 Tutorial lecture

Solid state potentiometric sensors with gold internal contact

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Ion selective electrodes with ion-selective polymeric membrane coated directly on a metallic conductor belong to the group of solid-state sensors. The elimination of the internal electrolyte solution from a sensor architecture was found to affect the electrochemical stability of this type sensors. This was explained by not well thermodynamically defined interface between the polymeric membrane and solid conductor. To overcome this problem, several approaches have been proposed, including an addition of lipophilic silver-ligand complexes or conducting polymers into polymeric films as well as an incorporation of intermediate layer between metal contact and ion-selective membrane. For the later approach, redox-active polymer layers, conducting polymers and redox-active self-assembled monolayers (SAMs) have been investigated as the intermediate phase.

This talk will cover the general principles of solid-state ion-selective sensors, with special emphasis on their appropriate architecture and the role of intermediate layers. It will be shown that the improvement of the stability of sensors can be achieved by the application of hydrophobic interface that exhibits reversible electron/ion transfer at the membrane/solid contact. The steps of chemical modification of transducers with gold internal electrode, leading to ion-selective sensors of improved stability, will be discussed.

11:50 Keynote lecture

Biphasic electrochemistry of ionic liquids deposited on the electrode surface

Marcin Opallo[1], Joanna Niedziolka[1], Ewa A. Rozniecka[1], Juliette Sirieix-Plenet[2], Laurent Gaillon[2]

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The room temperature ionic liquids (RTIL) already plays important role as electrochemical solvents. However biphasic electrochemical studies involving RTIL being in contact are rather scarce. This is despite their importance in extraction separation processes Here the results of the electrochemical studies of a redox probe dissolved in RTIL droplet deposited on the gold electrode immersed in an aqueous solution will be presented presented. It is shown that electrode reaction involves heterogeneous electron transfer followed by cation ejection to the aqueous solution or anion insertion to RTIL phase. The contribution of the latter process is judged from the salt concentration dependence of redox potential (E^0) and anion dependence of the same parameter. It has been concluded that for most hydrophobic RTIL 1-decyl-3-methylimidazolium bis(trifluoromethyl- sulfonyl)imide insertion of less hydrophilic anion is electrogenerated. In the case of droplet of less hydrophobic 1-butyl-3-methylimidazolium (bmim) hexafluorophosphate and bis(trifluoromethylsulfonyl)imide ejection of bmim$^+$ cation dominates. This behaviour is similar to that observed for drop or droplets undiluted and diluted organic liquid. The order or transfer potentials of anion across RTIL-water interface is similar to that observed for nitrobenzene-water interface. The electrochemical behaviour of analogous system based salt solution in hydrophobic solvent (2-nitrophenyl octyl ether) will be also presented and compared.

12:10 Short communication

Ion transfer electrodes based on carbon nanofibers embedded in silicate film modified with room temperature ionic liquid

Ewa A. Rozniecka[1], Joanna Niedziolka[1], Juliette Sirieix-Plenet[2], Laurent Gaillon[2], Maria A. Murphy[3], Frank Marken[4], Marcin Opallo[1]

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Biphasic systems composed of room temperature ionic liquids (RTILs) being in direct contact with an aqueous phase received recently some attention. This is because of their ability to extract ions or neutral molecules from the aqueous phase and therefore their potential application in extraction or liquid-liquid separation [1,2].

Among other driving forces the ion transfer across RTIL - aqueous solution interface can be driven by electric charge generation within RTIL during the electrooxidation of redox probe dissolved in this phase [3]. If RTIL is very hydrophobic this electrochemical process generates anion transfer from aqueous phase to RTIL [3]. To realise such system in practise the development of electrode support exhibiting strong affinity towards RTIL and repelling properties towards the aqueous phase is desirable. This can be done by application of heterogeneous conducting material combining above properties. Recently we have shown that carbon - hydrophobic silica composite material can be used for this purpose [4]. Since this material cannot be employed for thin film preparation we propose to use for this purpose hydrophobic carbon nanofibers (CNF) - silica film deposited on the electrode surface [5].

In this work we have attempted to immobilize microphases or thin film of redox probe (tert-butylferrocene, tBuFc) solution in the hydrophobic ionic liquid (1-decyl-3-methyl-imidazolium bis(trifluoromethyl-sulfonyl)imide, C10mimN(Tf)2) on CNF-silica film modified ITO electrode.

The electrochemical properties of these electrodes were investigated using cyclic voltammetry and differential pulse voltammetry in the aqueous solution of wide range of anions. The shape of the voltammogram and the current magnitude depends on the type of electrode surface and the form of the ionic liquid deposit. The anion effect on the ability of tBuFc molecules to be electrooxidized is observed. The presence of the hydrophobic CNF-silica film enhances the magnitude of the current.

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12:25 Short communication

Nanoobjects embedded in silicate films for biphasic electrodes

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Liquid | liquid ion transfer processes can be observed at electrodes with an immobilised liquid phase [1-3]. Therefore the development of suitable electrode support is highly desirable [4,5]. Two novel types of electrode surface modification based on conducting ITO nanoparticles or carbon nanofibres embedded in a hydrophobic silicate film are presented. The first method employs layer-by-layer deposition of the ITO nanoparticles onto an ITO support. This procedure is followed by sol-gel deposition of a hydrophobic methyltrimethoxysilane (MTMOS) film. The second method involves deposition of a film of a carbon nanofibres (CNF) - silica nanoparticles suspension in a MTMOS sol. AFM and FEGSEM images demonstrate the presence of porous ITO nanoparticles or CNF films. The porous surfaces gives a unique and highly conducting matrix for redox liquid immobilisation. After modification of these electrodes with t-butylferrocene or cobalt (II) tetraphenylporphyrin in phenylpropylpyridine, their electrochemical behavior in the presence of aqueous electrolyte media was examined. The current for the conversion of redox active compounds is increased by two orders of magnitude in comparison to electrodes based only on hydrophobic silicates. Therefore a high efficiency of the liquid | liquid ion-transfer electrode process is possible.

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[2] F. Scholz, R. Gulaboski, *Chem. Phys. Chem* ., **6** (2005) 16.
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[4] J. Niedziółka, M. Opallo, *Electrochem. Commun*., **6** (2004) 475.
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12:40 Short communication

Oxygen electroreduction by fungal laccases - combination of electrochemical and spectral data

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In order to investigate the mechanism of oxygen electroreduction by fungal laccase combined as well as stand alone spectral and electrochemical approaches have been exploited. Several homogeneous preparations of laccases from different basidiomycetes have been used, which were recently biochemically characterized in detail [1]. Currently it is believed that the redox potentials of the T2 and T3 copper sites in laccase are very close to each other. These potentials are approximately equal to 400 mV in low redox potential laccases and to about 800 mV in high potential laccases [2]. For the first time we report [3] that the redox potential of the T2 copper site of a high redox potential laccase from the basidiomycete *Trametes hirsuta* is close to 400 mV *vs*. NHE. Electrochemical studies of laccases from different sources show that these enzymes establish different heterogeneous ET pathways on carbon and gold electrodes [3-5]. It is determined that in the case of carbon electrodes a "normal" ET pathway can be observed, i.e., electron donor (electrode or substrate) - T1 site - T2/T3 cluster [4,5]. Due to this reason laccases adsorbed on carbon electrodes usually show well-pronounced electrocatalytic reduction of oxygen [4,5]. However, in the case of gold electrodes the heterogeneous ET pathway from the electrode to the copper centres in the enzyme globule is different [3,5]. We found that *Trametes hirsuta* laccase on gold electrodes electronically communicate with the electrode surface through the T2/T3 cluster. We observed that the electrons from the electrode to the T1 copper flow through the T2/T3 coppers. We confirm that T1 site has a redox potential of 780 mV [1,3,5], however, the redox potential of one copper ion from the T2/T3 cluster is close to 400 mV [3,5]. From the data about direct ET reactions of laccase on carbon and gold electrodes, as well as from the spectral results a possible modification of the catalytic cycle [6] of the enzyme is suggested and will be discussed.

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Acknowledgement: This work was financially supported by the European Commission (ICA2-CT-2000-10050) and the Swedish Research Council. The Swedish Institute (SI) is acknowledged for the support of a postdoctoral fellowship for Sergey Shleev.

Lunch

Wednesday afternoon, 9 November, 12:55

SESSION 9

Chairpersons: Gary Blanchard, Paweł G. Krysiński
Wednesday afternoon, 9 November, 14:20

14:20 Tutorial lecture

Extraction of local kinetic information from SECM approach curves to enzymatically active materials

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Scanning electrochemical microscopy (SECM) has become an indispensable tool for the characterization of reactivities at interfaces such as reactions of immobilized enzymes and other biomolecules. The analysis is based on the detection of redox active species generated by an immobilized enzyme though conversion at the scanning ultramicroelectrode (UME).

When imaging surfaces of sensors some special attention has to be made for correctly designing the experiments to avoid artefacts from other side reactions. This will be illustrated by several examples.

Recently, very much effort has to be devoted to extracting rate constants fro various reactions from approach curves to different materials. This can be done either in the feedback mode or in the generation collection mode. The talk will discuss, when the modes are appropriate and give a special instruction how carry out the analysis in feedback mode. Attention must be paid to three stages of the analysis: precise measurement of the curves and the control experiments, data treatment to extract the pseudo-first order rate constant and kinetic analysis of more complex reaction mechanisms to relate the pseudo-first order constant to more complex reaction mechanisms.

15:00 Keynote lecture

New electrode surfaces for solid | liquid | liquid triple phase boundary electrochemistry

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Processes at electrode surfaces strongly depend on the nature of the solid | liquid (electrolyte) interaction. This is also the case for processes at solid | liquid | liquid line interfaces and demonstrated here for the case of electrochemical processes at electrode | organic liquid | aqueous electrolyte triple phase boundaries.Experimental data are obtained at metal, carbon, and ceramic electrodes and for porphyrinato metal complexes immobilised in water-immiscible organic liquids in contact with an aqueous electrolyte [1]. Voltammetric data are discussed and the porosity of surfaces identified as a key parameter for optimising electrochemically driven ion transfer processes. Both the transfer of inert ions and facilitated ion transfer are considered.

[1] F. Marken, K. J. McKenzie, G. Shul, M. Opallo, *Faraday Discussions*, **129** (2005) 219.

15:20 Keynote lecture

Elektrochemiluminescence onto conducting polymer films for the bio-detection in DNA-chips

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Electrochemiluminescence (ECL) is widely used in analytical chemistry and shows several advantages, such as high sensibility and selectivity. ECL is particularly well adapted to the biochip based on the immobilization of DNA probes onto a conducting polymer layer, such as the polypyrrole (Ppy). This bio-sensitive layer was electrogenerated on the electrode surface from pyrrole units and pyrrole monomer bearing DNA. The recognition between the DNA probes onto the Ppy and DNA targets present in an analyte could be achieved by ECL. The principle of this detection method is, as follow. Once the hybridization (DNA probes)/(DNA targets) is completed, the ECL marker was immobilized to the DNA targets thanks to the biotin-avidin interaction, for example. Then, the electrochemical generation of the ECL reaction is induced by polarization of the electrode coated with the DNA functionalized polypyrrole film. ECL combines the advantage of the straightforward monitoring of the electrical addressing with the sensitivity of the luminescence.

Various markers could be used as ABEI (luminol derivative) or $Ru(bpy)_3^{2+}$ (bpy=2,2'-bipyridine). In a first step, the results shown in this communication concern the usefulness of the ECL reaction of ABEI in solution generated onto the surface of the electrode coated by a polypyrole film. Our results demonstrated that the potential value to activate the ECL reaction must be in a potentiel range where the polypyrrole matrix is or remains conducting for the time required for the ECL reaction to take place. In a second step, the detection of hybridization DNA probes/DNA targets from ECL of ABEI anchored to DNA targets was studied from a biochip format. The results achieved show that it is possible to discriminate the ECL emission generated from the duplex to this one corresponding to the background signal. In the last part, the ECL of $Ru(bpy)_3^{2+}$ was studied onto polymers as polypyrrole and polythiophene showing that this marker could be used for the DNA recognition in a bio-sensitive layer of conducting polymer film as an underlayer.

15:40 Short communication

Activation of voltammetric response of two-component film of functionalized fullerenes bearing a redox probe and palladium

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Electronically conductive carbon materials are known as effective electrode materials for electronic, electrochemical, and analytical applications. The electronic properties of these materials depend on their oxidation state. Hence, reversible addition and abstraction of electrons, and switching process from a non-conducting to a conducting state are important factors for these materials to be useful. The oxidation or reduction of the material generates electronic conductivity inside the polymeric phase. Different factors, including the degree of solvent swelling, supporting electrolyte, and the structure and morphology of the film are known to affect the process of conductivity switching which also determine the redox properties of the materials.

The electrochemical reduction of a solution containing a fullerene, generally C_{60}, and various transition metal complexes results in the formation of electrochemically active films on the electrode surface. In these films, fullerene moieties are covalently bound to transition metal atoms or their complexes to form a polymeric network. The two-component films, C_{60}/Pd and C_{60}/Pt, prepared from C_{60} and palladium or platinum containing precursors are particularly interesting. These films can be readily prepared with relatively high yields. They exhibit high electrochemical stability over a large potential range. Electropolymerization of 2-ferrocenylfulleropyrrolidine (Fc-C_{60}/Pd), a fullerene with a covalently appended ferrocene, in the presence of palladium and platinum complexes also produces films.

In this presentation, we report the electrochemical properties of new two-component films formed from a zinc porphyrin appended fullerene and palladium acetate and compare this new type of film with the films formed from C_{60} and palladium (C_{60}/Pd), 2-ferrocenylfulleropyrrolidine and palladium (Fc-C_{60}/Pd). The redox-active films were formed in solution containing a large excess of the palladium acetate precursor which lead to the deposition of palladium particles onto the growing polymer. These films undergo a significant change in redox activity when polarized at positive potentials.

15:55 Short communication

Room temperature ionic liquid based carbon paste electrode

Galyna Shul[1], Ewa A. Rozniecka[1], Marcin Opallo[1], Juliette Sirieix-Plenet[2], Laurent Gaillon[2]

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The carbon paste electrodes (CPEs) have been employed in a variety of applications such as analysis of numerous organic and inorganic compounds. Typically, the body of CPE is composed of graphite particles and non-conducting non-polar organic liquid like Nujol, silicone oil or high liquid hydrocarbon [1, 2]. One of the way of CPE modification is the dissolution of a redox probe in a binder [2]. Recently, we have presented electroactive CPE prepared with the redox probe solution in the organic polar solvent as the binder [3]. The structure of CPE, where small aggregates of graphite particles and polar organic phase in contact with an aqueous solution, give possibility to formation of three-phase junction: graphite particle / polar solvent / aqueous solution.

We will present new type of elecroactive CPE with conducting binder (room temperature ionic liquid, RTIL). It exhibits electrochemical signal connected with electrode reaction of the redox probe (*tert*-butylferrocene) dissolved in RTIL. The efficiency of the electrode process is much larger than that of similar CPE modified with the redox probe solution in less viscous organic polar solvent. It could be connected with the presence of conducting organic phase, and as a consequence with the enlargement of reaction zone from the three-phase junction to two-phase junction: graphite particle / RTIL. Moreover, the midpeak potential depends on the hydrophobicity of the anion present in the aqueous solution indicating significant contribution of anion injection into the ionic liquid following the electron transfer. In addition, this electrode exhibits sensitivity towards the concentration of anion in the aqueous phase.

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Coffee break/Poster session

Wednesday afternoon, 9 November, 16:10

SESSION 10

Chairpersons: Paweł J. Kulesza, Francis D'Souza
Wednesday afternoon, 9 November, 16:35

16:35 Tutorial lecture

Wiring of whole living bacteria with osmium-redox polymers

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Mediated electron transfer from microbial systems to electrodes represents a promising alternative to Clark electrodes. Electron mediators perform a special function in whole cell biosensors; their role is to replace the electron transfer performed by oxygen, thus preventing the process from the problem of having a low oxygen concentration [1]. Since the first applications of osmium redox polymers for enzyme based reagentless mediated biosensing were described, polymeric mediators still attract attention due to the efficient electron shuttling properties combined with the polymeric structure promoting a stable adsorption as well as a possibility for multiple layers of both immobilised enzymes [2] and microbial cells [3] on the electrode surface.

In the present study applications of flexible $Os^{2+/3+}$ functionalised polymers such as poly(1-vinylimidazole)$_{12}$-[Os(4,4'-dimethyl-2,2'-dipyridyl)$_2$Cl$_2$]$^{2+/3+}$ and poly(vinylpyridine)[Os(N,N'-dimethyl-2,2'-biimidazole)$_3$]$^{2+/3+}$ for efficient electrical wiring of bacterial cells is described. The methylated biimidazole complex was recently reported as a fast redox mediator due to its long tethering to the polymer backbone through a 13-atom-long flexible spacer [4] and also shown to more efficient than other Os-polymers having redox potential values 250-300 mV more positive. *Gluconobacter oxydans* and *Pseudomonas putida* DSM 50026 were used as the biological components serving both as model bacteria but also for possible future applications in biosensing and biofuel cells.

[1] P. Skladal, N. O. Morozova, A. N. Reshetilov, *Biosens. Bioelectron.*, **17** (2002) 867.
[2] A. Heller, *J. Phys. Chem.*, **96** (1992) 3579.
[3] I. Vostiar, E. E. Ferapontova, L. Gorton, *Electrochem. Commun.*, **6** (2004) 621.
[4] F. Mao, N. Mano and A. Heller, *J. Am.Chem. Soc.*, **125** (2003) 4951.

17:15 Keynote lecture

Detection of anti-cancer agents using a competitive nucleic acid-on-chip assay

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Progress of industrial processes and medical diagnostics increases need for fast and easy handling methods for high-throughput recording of data, without complicated sampling. Different versions of a fluorescence based method using dsDNA and a specific nucleic acid intercalating dye has already been reported [1, 2]. The detection sensitivity of the described method was due to the competition between the dyes and the target analytes for binding to the nucleic acids. Different sequences of the oligo nucleotides have been studied; the result indicated that the sequence differences cause different binding ability to the dye or target sample. The interaction was studied by coating a thin-layer of oligonucleotides on a chip and supplying the mixture of the dyes and target sample. The competitive effects between the dye and the analyte were observed through the fluorescence signal decrease. A high sensitivity CCD camera was applied to perform the fluorescence imaging, which has the advantages of rapid measurement and high-throughput.

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17:35 Keynote lecture

Application of ZP chemistry for the immobilization of polycyclic aromatic hydrocarbons and enzymes on gold, boron doped diamond, indium tin oxide and quartz.

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Controlling the molecular structure and organization of interfacial region between solid substrate and electrolyte solution has become a routine in various domains of chemistry [1,2]. In this paper we show the formation of monomolecular layer of pyrene, derivatized with alkyl carboxylic acid side chains of various lengths and terminal functionalities, on the surface of gold, boron-doped diamond (BDD), indium-tin oxide (ITO) and quartz by means of zirconium phosphonate (ZP) chemistry. The choice of pyrene ring systems was based upon their use as optical probes of polarity and viscosity of local environment. Moreover, these molecules are electroactive and this enabled us to evaluate the surface coverage with these molecules. The ability to covalently bind such probes to variety of surfaces, including boron-doped diamond, due to its excellent electro-optical and chemical properties, will broaden the field of relaxation dynamics studies of these molecules. We have used steady-state fluorescence emission spectroscopy and electrochemical techniques to study the behavior of monomolecular films formed on different surfaces. We show that surface modification is possible on both hydrated oxide surfaces (ITO and quartz) as well as on oxidized carbon substrates (BDD). The latter case is especially interesting since BDD is widely believed to be inert and thus difficult to modify. Such modified substrates were subsequently used to immobilize laccase, preserving catalytic activity of this enzyme.

Acknowledgement: The authors are grateful to Professor Greg Swain, Chemistry Dept., Michigan State University, USA, for his generous gift of BDD thin films. This work was supported by the Ministry of Scientific Research and Information Technology in 2004-2007, Project No. PBZ 18-KBN098/T09/2003. The NSF-PAS mobility grant (PK) is also acknowledged.

17:55 Short communication

Electrocatalytic reduction of dioxygen by redox mediator and laccase immobilised in silicate thin film

Izabella Zawisza[1,2], Marcin Opallo[2], Jerzy Rogalski[3]

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Laccase from *Cerrena unicolor* was immobilised in thin silicate film deposited on gold electrode. This film was obtained by sol-gel process from tetramethoxysilane - 0.01 M phospahte buffer based sol containing dissolved enzyme. The ezyme imbeded into the hydrophilic silicate matrix exhibit catalytic activity towards dioxygen reduction to water in the presence of mediator: 2,2'-azino-bis-(3-ethylbenzothiazoline-sulfonic acid) (ABTS).

This catalytic effect is pH and temperature dependent. The highest performance of the enzyme is found in solution of pH 4.5 ± 0.5. The catalytic behaviour of the system depends also on time elapsed after electrode immersion into buffer solution. The higher pH the longer time is requred to observe maximal catalytic activity of laccase towards dioxygen reduction. In solution of pH 4.5 the maximal activity is obatained in 24 hours. In more basic solutions time elapsed to obtain highest activity increases to 70 - 120 hours.

The maximum activity of the enzyme was found in teperature range 40ºC - 65ºC. The temperature dependence of the catalytic current can be described by Arrhenius equation in temperature range (15 - 40ºC). However, at temperature higher than 50ºC deviations form Arrhenius plot probably because of enzyme denaturation.

18:10 Short communication

Mathematical tools for characterization of spatial variability of surface activity based on SECM images

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Scanning Electrochemical Microscopy (SECM) has been successfully used for the imaging of modified surfaces revealing localized chemical information such as immobilized enzyme activity, electron-transfer kinetics etc. [1]. Previously, we have used enzyme microstructures visualized by SECM for compensating for potentially interfering compounds or determination of more than one substrate [2].

It is usually considered that by integration of many differently modified structures the amount of information about a measured sample can be significantly increased as compared to using a single structure. Sensor arrays usually consist of a number of individually and by purpose differently modified sensor surfaces, thus potentially allowing for the evaluation of complex multi-component samples. In contrast, each defined surface area modified with a sensing chemistry exhibits spatial inhomogeneity and could hence be treated similar to a sensor array, provided the possibility to extract localized information.

In order to demonstrate this approach enzyme/polymer layers were fabricated on surfaces and the localized sensor response upon addition of the enzyme's substrate was visualized with high lateral resolution using SECM, in the generator/collector mode (Fig. 1).

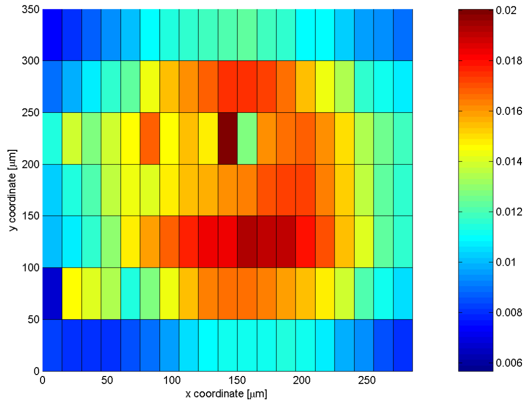

Fig. 1 SECM image of the localized sensor response [nA] of an enzyme/polymer
microspot in the presence of enzyme's substrate.

The SECM images obtained at different substrate concentrations were used as a basis for investigation of spatial variability of surface activity with feature extraction techniques.

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[2] S. Gaspar, M. Mosbach, L. Wallman, T. Laurell, E. Csöregi, W. Schuhmann, *Anal. Chem.,***73** (2001) 4254.

Special brake for growing more hungry

Wednesday evening, 9 November, 18:25

Dinner/Grill

Wednesday evening, 9 November, 18:45

Thursday, 10 November

Breakfast

Thursday morning, 10 November, 8:00

SESSION 11

Chairpersons: Gunther Wittstock, Renata Bilewicz
Thursday morning, 10 November, 9:00

9:00 Tutorial lecture

Reagentless biosensors based on mediator-modified electrodeposition hydrogels

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Recently, the electrochemically-induced selective modification of electrode surfaces by means of electrodeposition hydrogels under simultaneous entrapment of biological recognition elements was reported [1]. This method is based on an electrochemically-induced pH-modulation within the diffusion zone caused by the oxidation or reduction of water concomitantly modulating the solubility of the polymer chains. This change in solubility causes the precipitation of the polymer exclusively on the electrode surface. In the presence of a suitable enzyme the biological recognition element is entrapped within the precipitating polymer film and by this securely immobilized on the electrode surface. The synthesis of electrodeposition paints enables on the one hand the adaptation of the properties of the deposited polymer film to the specific needs of the entrapped biological recognition elements. On the other hand, specific functionalities may be integrated for further polymer crosslinking, attachment of redox mediators etc. The tutorial lecture will be directed to the specific considerations in the development of reagentless amperometric biosensors. Especially, the design of electron-transfer pathways between polymer-entrapped redox proteins and the electrode surface will be discussed in detail [2]. Based on this background, recent work concerning the application of redox-modified electrodeposition hydrogels will be described and the points will be discussed in detail: (i) synthesis of monomers for the coordinative binding of metal complexes; (ii) synthesis of libraries of electrodeposition paints and their modification with redox relays; (iii) voltammetric characterization of the obtained redox polymers; (iv) possibilities for varying the redox potential of the redox hydrogel; (v) automatic optimization of related reagentless biosensors using an automatic electrochemical robotic system; (vi) integration of PQQ-dependent dehydrogenases within redox relay modified electrodeposition paints; (vii) multi-layer multi-enzyme biosensors.

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Acknowledgement: Financial support from the EU (INTAS 01-2065 and INTAS 03-51-6278) is acknowledged.

9:40 Keynote lecture

Electrochemically formed fullerene-based polymers

Krzysztof Winkler[1], Wlodzimierz Kutner[2], Alan L. Balch[3]

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New materials based on electrochemically active polymers are continuously being developed for numerous applications. They can be used in electrotechnology, electrocatalysis and electroanalysis. From this point of view, fullerene-based polymers are very attractive. Up to six, reversible, one-electron steps are observed for the reduction of C_{60} in solution. Fullerene cages incorporated into polymeric structures are expected to retain this sort of electrochemical activity.

Electrochemical methods were also used for forming fullerene-based polymers and their immobilization on the electrode surfaces. These techniques allow control the amount of polymer deposited, its morphology, and its oxidation state. Electrochemically produced polymeric systems can be divided into four groups: (i) "*charm bracelet*" polymers formed from the electrochemically active fullerene derivatives, (ii) ionically induced polymers, (iii) fullerene epoxide based polymers, and (iv) "*pearl necklace*" co-polymers of fullerenes and transition metal complexes. In this presentation, special emphasis is placed on the electrochemically formed fullerene epoxide based polymers and polymers of fullerenes and transition metal complexes.

Polymeric films are formed during electroreduction of epoxide $C_{60}O$. It has been postulated that the electrochemically formed $C_{60}O^{2-}$ anion initiates the polymerization process. In the polymeric network, C_{60} cages may be connected through covalent C-O-C linkages although direct C-C linkages are also likely to be involved. Related films are readily prepared by the reduction of C_{60} and C_{70} in the presence of limited amounts of dioxygen in a toluene/acetonitrile mixture.

A two-component electroreductive process that involves the reduction of C_{60} (or a C_{60} derivative) in the presence of transition metal complexes of Pt, Pd, Ir, and Rh has been also developed. The fullerene/transition metal films that result are particularly interesting. In these systems, the polymeric network is believed to be formed through covalent bonding between transition metal atoms (or complexes) and fullerenes.

10:00 Short communication

Visualisation of the effect on catalytic activity of increasing Pt agglomerate sizes using constant-distance mode SECM in a competition mode

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In the area of Fuel Cell research the catalyst of choice for the Oxygen Reduction Reaction (ORR) is Pt on a C substrate. However the main problem with this and one of the stumbling blocks in making Fuel Cells economically appealing is the high cost of this catalyst. Thus, if it were possible to obtain the required surface activity to maximise the ORR but with less Pt present then the cost of fuel cell production would decrease dramatically. To gain an overview of the overall effect of the Pt agglomerate size on the electrocatalytic activity a study was conducted using high-resolution SECM.

A library of Pt agglomerates was created on a glassy carbon substrate allowing the determination of the effect of the Pt surface area and the size of the formed nanoclusters on the ORR. Analysis was conducted using two similar techniques. The first technique involved scanning the Pt agglomerate with a microelectrode SECM tip with the application of a potential step profile and recording the current with and without an applied substrate potential. The electrochemical images from this study produced information on the catalytic performance of each of the Pt agglomerates. The second technique involved the visualisation of each Pt agglomerate using constant-distance mode SECM in combination with a second potential pulse program. In this pulse program O_2 is firstly produced at the tip then subsequently reduced in the presence and absence of an applied substrate potential providing O_2 reduction competition between tip and sample. This competition reaction is used to elucidate electrochemically the effect of the Pt agglomerate size on the amount of oxygen reduced. The results from this combination of constant-distance mode SECM and O_2 reduction competition reaction provided a detailed image of each of the Pt agglomerates with high spatial resolution combined with a detailed view of the distribution of electrochemical activity over the agglomerate's surface.

Acknowledgement: Financial support from the MWF-NRW "Nachwuchsgruppe Brennstoffzelle" is acknowledged.

Coffee break/Poster session

Thursday morning, 10 November, 10:15

SESSION 12

Chairpersons: Paul A. Millner, Wojciech Macyk

Thursday morning, 10 November, 10:35

10:35 Keynote lecture

Detection chlorpyrifos oxon using screan-printed electrodes with difference acetylocholinesterases

Tomasz Sikora[1], Marek Trojanowicz[1], Sandra Eremia[2], Jean-Louis Marty[3], Adina Plotogea[4]

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The aim of this work is to presents an amperometric biosensor on the inhibition of cholinesterase (AChE) for detection of organophosphorus (OP) pesticides. Because organophosphorus pesticides have low persistent in the environment they are used for agriculture purpose but because they posses high toxicity they disturb the ecosystems. For environmental monitoring, the interest was to create more sensitive and selective methods, which promise simple and rapid measurements for analysis many pollutants. A reason was to create an faster detection methods capable to identify the contaminated samples.AChE got natural high sensivity for insecticides. The wild type and mutant enzymes B394 were tested against insectiside - chlorpyrifos oxon. The genetical modyfication of the enzymes increase their sensivity for insectisides. To determination constant of inhibition it was used the spectrophometric measurement of activity of enzyme using Ellman method for few different concentrations of pesticide. The best enzyme was used to made the electrodes. The AChE was immobilized on the surface working electrode (WE) using PVA. Screen-printed electrodes (SPEs) were prepared in a three-electrode configuration.For those electrodes were tested stability and percentage of inhibition. Amperometric measurements were performed in magnetically stirred solutions. The applied potential was of 100 mV.

10:55 Short communication

New approaches of invertase immobilisation on functionalised support-nanoparticles and films

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The experiments were focused on two directions: immobilisation of invertase on new supports based on chitosan and hyaluronic acid ionically cross-linked and on magnetic nanoparticles covered with various polymers.

Biopolymeric films were obtained by mixing different ratios of 2% (w/v) chitosan and 1% (w/v) hyaluronate solutions. Chitosan/hyaluronic acid films were cross-linked with sodium citrate by soaking chitosan/hyaluronic acid films into a sodium citrate solutions (2.5, 5%, w/v). Immobilisation of the enzime on the film surface was made through immersion. The ultrastructure of carried out films (chitosan, chitosan/hyaluronic acid and invertase immobilised on chitosan/hyaluronic acid film) were exmined using Confocal Scanning Laser Microscopy.

Magnetic nanoparticles were synthetized by thermal co-precipitation of ferric and ferrous chloride. In order to improve biocompatibility, magnetic carriers were encapsulated using polyvinyl alcohol, polyethylene glycol, polyvinyl acetate and polyacrilic acid as sheel materials. Enzyme was immobilized on the functionalized surface. Transmision Electron Microscopy and Scanning Electron Microscopy were used to characterize the particles, having a size between 18 nm and 580 nm. Fourier Transformed Infrared Spectroscopy was used to confirm the structure of functionalized particles and also the enzyme binding, via monitoring the changes in shape, intensity and wavelength shift (1715.5 - 1732 cm^{-1}) of the - COOH group of polymers.

The study indicates that the stability and activity of enzyme were enhanced via magnetic nanoparticles and films binding.

11:10 Short communication

Poly-(m-phenylenediamine, resorcinol) and Prussian Blue chemically modified transducer for improving long-term stability and selectivity of the oxidase-based biosensors

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Amperometric biosensors with H_2O_2 detection are important and widely used analytical systems. In case of long lifetime and stability of immobilised biolayers, the electrode system becomes the limiting factor for the long-term biosensor use. This is associated with the working electrode surface passivation and thus decreased real polarisation voltage on the active surface. In this study, a chemically modified transducer with poly(*m*-phenylenediamine, resorcinol) / Prussian Blue / Pt electrode was prepared via electrodeposition of Prussian Blue as electron transducer followed by electropolymerisation coating by a selective diffusion layer of *m*-phenylenediamine and resorcinol. This system was compared with the bare Pt electrode. Both systems were designed as glucose biosensors by immobilisation of glucose oxidase as a top layer of the sensor. Ag/AgCl was used as a reference electrode. The long-term stability and response in dependence on the polarisation voltage was studied. The influence of interferents was also evaluated. The possibility of extending this system into multichannel configuration was tested and suitable methods of enzyme layer were developed and compared.

11:25 Short communication

Aging and biocomplexity: fractal analysis in the diabetic retinopathy as a model for the study of complex physio-pathological structure

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Retinopathy represents the biggest cause of blindness in the industrialized world, with a high incidence for old people. It point's out all the consequent affection of the retina to systemic illnesses as: the diabetes (diabetic retinopathy), the hypertension (hypertensive retinopathy), other disorders of endocrine nature and local degenerative phenomenon or metabolic functional, connected with aging.

The main purpose of the project is to

- develop a software able to qualify the abnormalities in the retinal vessel network, using highly sensitive parameters to pathology progression;

- deepen the employment of the fractal geometry within the identification and the qualification of the diabetic retinopathy;

- make an automatic method of segmentation of the vascular structure and to study the possibility to use it in substitution to the manual method;

- make a procedure for the extraction of the vascular retinal pattern and to follow the retinal vessel system modification using fractal dimension.

The current version of the method has been tested on several images differing for local retinal pattern view and ocular background.

The obtained results show that retinal vascular system is a fractal. The fractal dimension values are coherent with the analyzed figure complexity.

Closing remarks

Thursday morning, 10 November, 11:40

Lunch

Thursday afternoon, 10 November, 12:00

Checking out

Thursday afternoon, 10 November, 13:30

Departure for Warsaw by bus

Thursday afternoon, 10 November, 14:00

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