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Gas Sensors
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Electronic Nose and odour recognition
Chair Dr.G. Di Francia - *CR-ENEA Portici*
Chair Prof.P.Pelosi - *Universita' degli Studi di Pisa*

Biosensors
Chair Prof. M. Mascini - *Università. di Firenze*
Chair Prof.C. Botrè - *Universita' degli Studi di Roma*
Chair Prof.G.C.Scibona - *Universita' degli studi della Tuscia*

Environmental Applications
Chair Dr. C. Cremisini - *CR-ENEA Casaccia*

Photosynthetic Biosensors
Chair Dr.M.T. Giardi - *CNR-IBEV Monterotondo*

Biosensors for heavy metal detection
Chair Prof.L. Campanella - *Universita' degli Studi di Roma*

Sensors for heavy metal detection
Chair Prof.G. Palleschi - *II[a] Universita' degli Studi di Roma*

Mass Production
Chair Dr.L.Pirozzi - *CR ENEA Casaccia*

ORGANIZZAZIONE

Franco Mazzei
Universita' "La Sapienza", DSCTSBA,
P.le Aldo Moro 5, 00185 Roma
E-mail: mazzei@uniroma1.it
http://www.uniroma1.it/bioprobe

Roberto Pilloton
ENEA, INN-BIOAG-BIMO, SP008
Via Anguillarese 301 - 00060 – Roma
E-mail: pilloton@mclink.it
http://inn7201.casaccia.enea.it

Segreteria Organizzativa
MariaRita Montereali
Tel.: 06-30483294 - Segr.Tel.: 06-30483814
Fax: 06-30483327
Biosensori@casaccia.enea.it

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O01

Thick film gas sensors based on nanosized semiconducting oxides

Giuliano Martinelli[1]*, Maria Cristina Carotta[1], Vincenzo Guidi[1], Cesare Malagù[1] and Enrico Traversa[2]

[1]INFM - Dept. of Physics, Univ. of Ferrara, Via Paradiso 12, 44100 Ferrara, Italy
[2]Dept. of Chemical Science and Technology, Univ. of Rome "Tor Vergata",00133 Rome, Italy

SnO_2, $LaFeO_3$, and $SmFeO_3$ thick films were prepared by screen-printing technology on alumina substrates with comb-type Au electrodes. An array of thick-film prototype sensors has been placed beside a conventional station for environmental monitoring. Field tests have been performed by measuring the change in conductivity of the thick films exposed to real atmosphere. Their electrical response has been compared with the results of the analytical instruments for environmental monitoring. The same trend was observed for both systems, with very promising results. This allows us to consider as feasible the use of these sensors for cheap, innovative stations for air quality control.

Keywords: thick film, semiconducting oxides, sensors.

*e-mail :martinelli@fe.infn.it

O02

Applications of Porous Silicon as a Gas Sensor

G. Di Francia[1], L. Quercia[1],G. Iadonisi[2], V. La Ferrara[2], L. Lancellotti[2], D. Ninno[2],C. Baratto[3],E. Comini[3], G. Faglia[3] and G. Sberveglieri[3]

[1]CR-ENEA Loc. Granatello, 80055 Portici(Napoli), Italy
[2]INFM, Dip. di Scienze Fisiche, Università di Napoli "Federico II"
Mostra d'Oltremare, Pad. 19, 80125 Napoli, Italy
[3]INFM Dip. di Chimica e Fisica dei Materiali, Universita' di Brescia, Via Valotti 9, 25133 Brescia, Italy

Porous Silicon has been the most investigated material over the last decade. The main aim of this work is to describe its potential applications as gas sensor discussing reported data and authors' experimental findings. Fabrication methods and material properties as well as the possible mechanisms of interaction with the environment are also discussed.

Keywords: sensor, porus silicon, nanophase.

O03

Surface Acoustic Wave Devices for Gas Sensing Applications

M. Penza

PASTIS-CNRSM S.C.p.A. SS. 7, Appia, Km 7+300 per Mesagne, 72100 Brindisi – Italy.

An overview of the Surface Acoustic Wave (SAW) gas sensors developed by PASTIS-CNRSM SCpA will be given. The gas sensing characteristics of these SAW gas sensor devices will be described with respect to transient response, calibration curves, response time, ageing, sensing mechanisms, cross-sensitivity, selectivity. It will be discussed the gas sensing properties of the used sensitive coatings, inorganic and organic, prepared by different process technology such as PVD systems, Langmuir-Blodgett, spin-coating techniques. Different SAW gas sensor configurations will be examined such as SAW delay line, resonator, oscillator, acoustic modes-transduction delay line, by indicating their main technical features and their gas sensing performances.

Keywords: surface acoustic wave gas sensors, sensor materials.

E-mail address: penza@cnrsm.it

O04

Olfactory receptors: rom cloning to function

Giacomo A.F. D'Agostaro

Dipartimento Ambiente, ENEA, Casaccia, Roma.

The olfactory system is able to discriminate a large array of odor molecules and to translate olfactory stimuli into complex sensory information. The initial stage in olfactory discrimination involves the interaction of odor molecules to specific receptors on the surface of olfactory sensory neurons. This ligand-receptor interaction initiates a cascade of signal transduction events that involves opening of ion channels, membrane depolarization, and generation of action potentials. These signals are transmitted to the olfactory bulb in vertebrates and to the antennal lobe in insects, where the axons from the olfactory neurons form synapes with the dentrites of secondary neurons and interneurons within structures called glomeruli. The secondary neurons integrate the inputs from the sensory neurons and relay the olfactory information to central areas of the brain.

The olfactory receptors (OR) were first identified in the rat, through cloning of their genes. Todate, OR genes have been isolated from several vertebrate species and, recently, from Drosophila. The identification of the OR genes has been instrumental in studying the molecular mechanisms of olfactory discrimination. This review summarizes the rapid advancement in our understanding of odor discrimination since the discovery of the OR gene family.

Olfactory recognition is mediated by a large number of receptors sharing a common membrane topology. Individual olfactory neurons express only one OR gene, so that neurons are functionally distinct.

Sensory neurons expressing a given receptor synapse at the same glomerulus in the olfactory bulb of vertebrates or the antennal lobe of insects. The pattern of convergence is invariant in all individuals of a species.

Cognate ligands of some OR have been positively identified, paving the way to a detailed molecular understanding of ligand-receptor interactions. The results of structure-function relationship studies should allow in the future the generation of OR endowed with new and predetermined selectivity.

Keywords: olfactory system, receptors

O05

Physiological and artificial systems for odour recognition

P. Pelosi[1], K. C. Persaud[2]

[1]Dipartimento di Chimica e Biotecnologie Agrarie, University of Pisa, Via S. Michele, 4, 56124 Pisa, Italy
[2]Department of Instrumentation and Analytical Sciences, UMIST, Manchester, UK

The design of an artificial nose requires two types of contributions: transducers, to convert chemical information carried by the odorant molecules into an electric signal, and information from the biological olfactory system on how chemical structures are associated to odours. The current availability of different types of gas sensors, fulfilling the required properties, and the increasing information on the biochemistry of olfactory transduction make such project feasible. Transducers based on conducting polymers are currently being employed in artificial devices for gas discrimination and recognition, currently applied to food headspace analysis, environmental monitoring and clinical diagnostic. The use of odorant-binding proteins of the olfactory system in the fabrication of biosensors for odours could in the future improve the performance of electronic noses.

Keywords: olfaction, structure-odour relationships, odorant-binding proteins, conducting polymers.

O06

Electronic Nose For Food And Other Applications

M. Pardo, G. Niederjaufner, E. Comini, G. Faglia, G. Sberveglieri

INFM, Gas Sensor Lab - Dept. of Chemistry and Physics for Engineering and for Materials, University of Brescia, Via Valotti 9 - 25133 Brescia -Italy

The problem of resolving similar food odors (similar cheeses and milks subjected to different heat treatments) with an electronic nose (EN) is addressed. The EN is constituted by an array thin films sensors, a static or dynamic headspace sampler and data analysis software (PCA and ANN in cascade). Further future applications, as for instance environmental monitoring, are also described.

Keywords: electronic nose, food analysis, thin films, sampling techniques, neural networks

O07

Metal oxide gas sensors prepared by sol-gel technology and their application in electronic-nose

P. Siciliano[1], R. Rella[1], L. Vasanelli[2], S. Capone[2], M. Epifani[2], A. Licciulli[3]

[1]CNR-IME Istituto per lo Studio di Nuovi Materiali per l'Elettronica, via Arnesano 73100 Lecce, Italy
[2]INFM - Università di Lecce, Italy
[3]PASTIS/CNRSM – Brindisi, Italy

Tin oxide thin films were prepared by the sol gel process and activated with Pd, Pt and Os for using as gas sensing devices. The films have been deposited by spin coating starting from hydrolysed alcoholic solution of tin precursors. The addition of inorganic transition metal salts in the solution enabled the activation of the resulting films. The electrical conductivity variations as a function of various gaseous atmospheres and temperature were measured to evaluate the sensing properties of the films. Among the others NO_2, CO, CH_4, CH_3OH and C_2H_5OH gases were used for the tests. Principal Component Analysis (PCA) was used as pattern recognition technique for the evaluation of the data coming from the array composed by the sensors, showing the use of sol-gel derived films as sensing devices to be used in electronic-nose.

Keywords: tin oxide, sol-gel, electronic-nose

O08
(presentazione virtuale)

Deposition of phtalocyanines and porphirins derivatives

L. Leo, G. Mele, G. Rosso, G. Stasi, L. Valli, G. Vasapollo

Dipartimento di Scienza dei Materiali, Università di Lecce,
Via Arnesano I, 73100 Lecce, Italy

Porphyrin-C_{60} has been prepared by 1,3 dipolar cycloaddition using *N*-Methyl-glicine and formylporphyrin with C_{60}. Cu-Phthalocyanine-C_{60} has been synthesised by reaction of 1,2 dicyano-4-(2', 4' di-*tert*-amyl-phenoxy)benzene with 1,2 dicyano-4-(4'-*N*-methyl-2-fulleropyrrolidine) in presence of $CuCl_2$ and DBU. The transfer of the porphyrin-C_{60} dyad onto solid substrates by the Langmuir-Blodgett (LB) method has been analysed.

Keywords: Non conducting electropolymerized film, sensors, biosensors.

O09

Electrochemical sensors and biosensors for the detection of doping substances and methods

Francesco Botrè[1], Francesca Buiarelli[2], Franco Mazzei[3]

[1]: Dipartimento di Controllo e Gestione delle Merci e del loro Impatto sull'Ambiente Università "La Sapienza", Roma
[2]: Laboratorio Antidoping, Federazione Medico-Sportiva Italiana, Roma
[3]: Dipartimento di Studi di Chimica e Tecnologia delle Sostanze Biologicamente Attive Università "La Sapienza", Roma

The detection of doping agents and of their metabolites in the athletes urines is generally performed by GC-MS techniques. These methods, although extremely powerful, require an extensive pretreatment of the urine, including a solid-liquid or liquid-liquid extraction step, enzymatic or chemical hydrolysis (when needed), preconcentration, and derivatization. While for the confirmation analysis chromatographic techniques with mass spectrometry detection still represent the unique analytical option (also from a merely normative point of view), electrochemical sensors and biosensors could represent a faster, simpler and more economical alternative for the preliminary screening analysis of doping substances and methods.

Analytical methods involving the use of electrodes and bioelectrodes for the detection of pharmaceuticals and their metabolites in biological fluids can be divided into three main classes:

1. combined chromatographic-electrochemical techniques, in which the electrochemical sensor or biosensor, assembled into a flow-through cell, constitutes the sensing element of the chromatographic detection unit;

2. stand-alone electrochemical or bioelectrochemical cells, where the detection unit is employed for batch measurements on a pre-purified fraction of the biological fluid (urine) to be assayed;

3. electrochemical immunosensors, where the immunological interaction between the sensor and the sample gives rise to a detectable change of a defined electrochemical parameter.

While the amounts of studies carried out on biosensors belonging to class 3 is still too limited to draw an even preliminary picture of the real potentiality of the relevant methods, sensors included in classes 1 and 2 have already been evaluated on real samples. More precisely, class

1 refers to HPLC methods with amperometric detection, whose advantage with respect to traditional HPLC-UV and also to GC-MS methods is given by a drastically simplified pretreatment procedure; while class 2 includes a wide variety of methods based on polarographic and voltammetric techniques, mainly adsorptive cathodic stripping voltammetry, cyclic voltammetry and differential pulse voltammetry.

An outline of presently studied methods is presented, focusing on those classes of doping substances (primarily β 2- agonists and corticosteroids) missing a reliable screening procedure in doping control analysis, as well as on specific compounds (e.g. some diuretics) whose detection by traditional GC-MS techniques can be affected by various experimental artifacts.
Depending on the specific class of compounds to be detected, the extent of the pre-purification process, the nature of the electrode and of the applied electrochemical technique, the lowest detection limit varies from 100-200 ng/ml down to few ng/ml, thus theoretically matching the sensitivity needed by an antidoping assay. The possibility of employing some newly developed electrochemical methods for the "in vivo" monitoring of biophysiological parameters strictly related to the athletic performance is also discussed.

Keywords: electrochemical sensors, voltammetry, doping analysis, beta agonists.

O10

Direct electrochemistry of membrane-entrapped horseradish peroxidase: amperometric detection of hydrogen peroxide

T. Ferri[1], A. Poscia[2], R. Santucci[3]

[1]Dipartimento di Chimica, Università di Roma 'La Sapienza' and Dipartimento di Medicina Sperimentale e Scienze Biochimiche, Roma, Italy
[2]Menarini Industrie Farmaceutiche Riunite SpA, Firenze, Italy
[3]Università di Roma 'Tor Vergata', Rome, Italy

In the last years, electrochemical biosensors (based on enzymes which catalize redox reactions) have found wide application for detection of a large body of biological substrates. The most recent amperometric biosensors, indicated as 'third-generation biosensors', do not make use of mediators; they monitor the presence of small molecules forming or disappearing during the reaction between an enzyme and the substrate (as the hydrogen peroxide formation or the oxygen disappearance) through the measurement of the current generated. The recent successfull employment of multienzymatic systems is mainly due to the ability to change the analyte in a form electrochemically detectable through a sequence of reactions, or eliminate intereferring substances by their convertion into inactive electrochemical compounds.
We describe here an amperometric biosensor based on horseradish peroxidase (HRP) entrapped within a tributylmethyl phosphonium chloride polymer bound (polystyrene crosslinked with 1% divynil benzene) anionic exchange resin at a pyrolytic graphite (PG) electrode. Data obtained indicate that the immobilized protein is electrochemically active within a wide pH range (pH 3.0-12.0) even in the absence of mediators, and shows catalytic activity in the hydrogen peroxide electroreduction. The ability of the system to acts as a biosensor has been tested by entrapping choline oxidase (or glucose oxidase) in the membrane together with HRP, and detecting the presence of choline (or glucose), respectively, in solution. The system revealed to act efficiently: to a good stability, it coupled rapid electron-transfer at the electrode and good biological selectivity. This system may represent a promising example of simple 'solid-state' sensor to be employed in electrochemical analysis of biomolecules in solution.

Keywords: electrochemical biosensor, horseradish peroxidase, hydrogen peroxide.

Acknowledgements - Work partially supported by grants from the CNR.

O11

Amperometric biosensors for food quality control. Determination of biogenic amines, lactulose and glycerol

D. Compagnone, D. Moscone, G. Palleschi

Dipartimento di Scienze e Tecnologie Chimiche, Università Tor Vergata
Via della Ricerca Scientifica, 00133 Roma, Italy

Amperometric biosensors for the measurement of biogenic amines in fish and fruit samples, of lactulose in milk and of glycerol in wine have been developed.
The covalent immobilization of enzymes as diamine oxidase (amines), b -galactosidase + fructose dehydrogenase (lactulose) and glycerokinase + glycerol-3-P oxidase (glycerol) has been carried out onto polymeric supports for the realization of enzyme membranes or onto glass beads for the realisation of enzyme reactors. Pt working electrodes were used as electrochemical trasducers. For most of the biogenic amines, lactulose and glycerol a linear response uo to 5×10^{-4} / 10^{-3} mol/L was obtained with RSD < 5%. Sensitivity was suitable for measurement in food samples. The biosensors were used to monitor biogenic amines production in salted anchovy samples, to measure biogenic amines content in modified amosphere packaged fruits, to determine lactulose in milk samples and to follow glycerol production during alcoholic fermentation. Recovery studies and/or comparison with reference procedures demonstrated that the biosensors can be used for food quality control.

Keywords: biogenic amines, lactulose, glycerol, food,amperometry

O12

Biosensori per lo studio dell'espressione genica: stato dell'arte

C. Marusic, E. Nebuloso e G. Giuliano

CR-Enea Casaccia

O13

Recent Advances on DNA Biosensors

S. Tombelli, G. Marrazza, M. Mascini

Dipartimento di Sanità Pubblica, Epidemiologia e Chimica Analitica Ambientale, Via G. Capponi, 9 50121 Firenze, Italia.

The determination of low-molecular weight compounds with affinity for DNA was measured by their effect on the oxidation signal of the guanine peak of calf thymus immobilised on the electrode sensor and investigated by chronopotentiometric analysis. The DNA biosensor is able to detect known intercalating and groove binding compounds. Detection limits of 0.3, 0.2, 10 mgl^{-1} were obtained for daunomycin, polychlorinated biphenils (PCBs) and aflotoxin B1, respectively. Applicability to river water samples was demonstrated.

Coupling of Polymerase Chain Reaction (PCR) with a piezoelectric biosensor for hybridisation detection, to detect a specific mutation in apolipoprotein E (apoE) gene has been realised. Biotinylated 23-mer probes were immobilised on the streptavidin coated gold surface of a quartz crystal; the protein was covalently bound to the thiol/dextran modified gold surface. The device was able to distinguish different synthetic oligonucleotides.

The hybridisation reaction was also performed using real samples of DNA extracted from human blood and amplified by PCR. The system was able to realise apoE typing distinguishing between different groups of genotypes.

Keywords: DNA, chronopotenziometry, piezoelectric biosensor

O14

Isolation of recombinant single-chain antibodies (scFvs) with desired specificity from a "single scaffold" phage display library

R. Franconi[1], A. Desiderio[1], P. Roggero[2] and E. Benvenuto[2]

[1]ENEA, C.R. Casaccia, INN BIOAG BIMO, Via Anguillarese 301, 00060, Rome, Italy
[2]CNR, Istituto di Fitovirologia Applicata (IFA), Strada delle Cacce 73, 10135, Turin, Italy

Antibody (Ab) fragments produced in bacteria may provide high specific, low cost reagents for immunodiagnosis. An antibody library, encoding a diverse array of synthetic Ab fragments, each displayed on the surface of filamentous bacteriophage, was obtained by randomisation of residues involved in the binding site of a recombinant single-chain variable fragment (scFv) antibody with intrinsic high stability. By using site-specific mutagenesis we have generated a library of mutant scFvs molecules with different specificity. We report the isolation of scFv antibodies specific to the plant virus cucumber mosaic (CMV). After four rounds of selection and enrichment ('biopanning') on the immobilised antigen (the viral coat protein), a panel of different phage clones were obtained. Fully active soluble antibodies were produced in *Escherichia coli* with high yields. These engineered Ab fragments represent a valuable tool for inexpensive diagnosis of CMV in infected plant extracts. Antibodies against virtually any antigen, including haptens, may be derived from this library, thus representing a repertoire of proteins with different binding activity for the development of new biosensors.

Keywords: phage display, scFv, diagnostics, biosensor

O15

Protein Immobilization on Solid Substrates for the Realisation of Optical Immunosensors

Claudio Domenici[1], Lorena Tedeschi[1], Arti Ahluwalia[1], Francesco Baldini[2], Claudia Preininger[2], Andrea A. Mencaglia[3]

[1]Istituto di Fisiologia del CNR, via Savi 8, 56100 Pisa, & Centro "E. Piaggio", Università di Pisa, via Diotisalvi 2, 56126 Pisa, Italy
[2]Istituto di Ricerca sulle Onde Elettromagnetiche "Nello Carrara", IROE-CNR, Via Panciatichi 64, 50127 Firenze, Italy
[3]Prodotec srl, Via Antognoli 28/30, 50127 Firenze, Italy

In order to optimise the performance of optical immunosensors, the sensitivity of active protein layers anchored to the optical surface has been analysed.
A comparative study of different immobilization techniques of antibodies on optical surface is presented. Planar substrates and polymeric coated fiber optic surfaces were analysed. In the former case antibody surface densities of the order of 300 ng/cm2 have been obtained, while in the latter the relative fluorescence of different coatings has been measured during the testing of the fiber optic sensor.
The effect of non-specific binding on the prepared surfaces has also been evaluated.

Keywords: immunosensor, antibody, surface, optical fiber.

O16

Recent Development of Environmental Sensors

L. Campanella, G. Favero, L. Persi, M.P.Sammartino, M. Tomassetti, G. Visco

University of Rome "La Sapienza", Chemistry Dept - Piazzale Aldo Moro, 5-00185 Roma, ITALY

The most recent developments in the electrochemical sensor and biosensor research and their applications to the environmental field performed in our laboratory are described. Particular attention is paid to the total toxicity determinations to the pesticide analysis and to the monitoring of inorganic ionic pollutants.

Keywords: sensors, biosensors, environment.

O17

Inhibition based biosensors: environmental applications

F. Mazzei[1], F. Botrè[2]

[1]Università degli Studi di Roma "La Sapienza" - Dipartimento di Studi di Chimica e Tecnologia delle Sostanze Biologicamente Attive
[2]Università degli Studi di Roma "La Sapienza" -Dipartimento di controllo e gestione delle merci e del loro impatto sull'ambiente

Kinetic assays involving inhibition of specific enzymatic systems have been extensively applied as analytical methods for the detection of food and environmental contaminants, primarily among them pesticides, herbicides and heavy metals.
A particular form of enzymatic inhibition assays is represented by enzymatic inhibition bioelectrodes, whose flexibility can ensure the analysis of huge population of samples at very reduced costs.
An "ideal" inhibition biosensor should, in principle, ensure the rapid detection of all contaminants endowed with the same biological effect, without the need for an extensive sample pretreatment.
In this work data are reported on the possibility to detect environmental contaminants endowed with toxic effects, and especially organophosphates and organochlorides compounds, widely used as pesticides. A new biosensor is proposed for the direct determination of 2,4 dichloro phenoxyacetic acid (2,4D), i.e. one of the most powerful and diffused defoliant, also endowed with estrogenic properties. The proposed biosensor could be very useful for its potential application on line or "on the spot".

Keywords: enzymatic inhibition, bioelectrode, environmental applications.

O18

OPEEs – what are they?

L. Campanella, G. Favero, M.P. Sammartino, M. Tomassetti

Department of Chemistry - University of Rome "La Sapienza" - P.le Aldo Moro, 5 - 00185 Roma ITALY

The catalytic activity of enzymes is often maintained in non-aqueous media, a possibility that is used to develop enzymatic electrodes working in organic solvent (OPEEs). Over the past few years, our research group have developed several OPEEs suitable for different applications: a catalase OPEE for the determination of hydrogen peroxide in pharmaceutical and cosmetic products, a tyrosinase OPEE for polyphenol analysis in olive oil, two bi-enzymatic OPEEs for the determination of lecithin or pesticides and a superoxide dismutase OPEE for free radical determination. Also a brief review of the best known and most popular OPEEs reported in literature, is presented.

Keywords: OPEEs, organic phase, enzymatic electrodes.

O19

Alternative methods to animal testing: the role of biosensors

Elisabetta Podestà, Bruno Silvestrini, Claudio Botré

Dipartimento di Farmacologia delle Sostanze Naturali e Fisiologia Generale
Università "La Sapienza" Roma

Increasing interest has been recently devoted towards the development of novel analytical methods to be used for the screening of various contaminants in the environment and in food products. For indeed, the usual analytical procedure which is followed to ensure the safety of food products is constituted by two stages: a preliminary testing (screening) of a representative population of samples to be assayed and the subsequent confirmation analysis of all samples which gave positive or even doubtful results after the first screening. It follows that the "ideal" screening method should ensure the detection of all different toxins of the same class (i.e. endowed with the same effect) without the risk of false negatives.

The only universally recognized (also from a strictly legal point of view) method for the screening of complex food products is represented by toxicity biotests, i.e. assays on animals (usually mammalians) in which the sample is either injected or mixed with food and the development of any toxic effect is monitored for a certain time interval (from few hours to several days). It appears evident that the development of valid alternatives to these methods would result in a drastic reduction of time and cost of analysis and would also virtually eliminate the need of animal sacrifice.

Biosensors would represent an adequate solution, since they lie in between the scale from physico-chemical tests (primalrily GC-MS and HPLC-MS), which are extremely specific and widely used as confirmation methods, and biological effect-based assays (primarily cellular toxicity tests or mammalian biotests), which are more universal and are used as a screening test.

This communication presents preliminary results obtained by bienzymatic, hybrid inhibition biosensors for the screening of algal toxins in seafood. Data refer to the detection of okadaic acid and its analogues (other toxins of the DSP - diarrheic shellfish toxin - group) in naturally contaminated samples: the same experimental approach could also be extended to the analysis of mycotoxins in coffee beans and derived products.

Keywords: toxicity tests, animal testing, bioelectrodes.

O20

Heavy metals and lactate monitoring system

M. Adami[1], G. Martinazzo[1], S. Villari[1], M. Panza[1], M. Sartore[1], C. Nicolini[2]

[1]Parco Scientifico e Tecnologico dell'Elba, 57030 Marciana (LI), Italy
[2] Istituto di Biofisica, C.so Europa 32, 16132 Genova, Italy

A recent research program at the PST Elba was focalized on the development of low-cost, portable electrochemical apparatus for the detection of analytes both in environmental and health care field. The instrument is capable to perform the detection in several matrices such as water (clear, sea, drinking, etc.), beverages and biological fluids. The operations are simple and completely computer-assisted by means of user-friendly menus. The operator is only requested to add the sample into the analytical cell, along with a small quantity of supporting electrolyte, and to set up a few measuring parameters. The system is completely automated and performs different electrochemical techniques, i.e. Potentiometric Stripping Analysis, Chronoamperometry and Linear, Cyclic, Differential Pulse and Square Wave Voltammetry. The working electrodes are graphite-based, eventually including the catalytic elements when required. Applications of the device in the detection of the presence of metal ions in solution, as well as of metabolites, are presented.

Keywords: metal ion detection, lactate detection, electrochemical instrument, electrochemical techniques.

O21

Fast and Sensitive Bioluminescent ATPase Assay for Heavy Metal Detection in Environmental Samples

N. Nedeljkovic[1], G. Nikezic[1], A. Horvat[2], M. Mirasoli[3], P. Pasini[3], A. Roda[3]

[1]Faculty of Biology, University of Belgrade, Yugoslavia
[2]Vinca Institute of Nuclear Sciences, Belgrade, Yugoslavia
[3] Università di Bologna, Dipartimento di Scienze Farmaceutiche

O22

A synthetic system mimics the Electron Transfer in Membranes: definition of the proteic scaffold

C. Cantale[1], M. S. Oriolo[1], M. Sperandei[1], M. Paci[2]

[1] ENEA INN/BIOAG-BIMO Via Anguillarese, 301, 00060 S. Maria di Galeria Rome, Italy
[2] Department of Chemistry, University "Tor Vergata", Via della Ricerca Scientifica, 00133 Rome, Italy

A great effort in the research world has been devoted to mimic the electron transfer of biological systems. The assembling of simplified proteic structures, molecular maquettes, with photosynthetic cofactors (chlorophylls, carotenoids, quinones) is considered a promising tool to gain relevant informations, allowing to study the main interactions, without the natural systems complexity.The pioneer works of DeGrado and coworkers (ranging from 1984-1995) have largely contributed in developping this approach, with a series of peptides binding up to four haem redox groups, which exhibit native-like properties in aqueous solution. We intend to carry out such an approach in simulated membrane systems (liposomes or Langmuir-Blodgett films). A *de novo* designed peptide was built, as the scaffold for a simple donor-acceptor system to be put inside a lipid film. A study of some wild protein structures in membranes was carried out, including structural motif as coiled coil and leucine zipper too, the signal peptides and the large complexes like the light harvesting and the photosynthetic reaction center, together with the statistics about the aminoacid propension in the different membrane portions. The designed peptide is 16 aminoacid long and it is planned to contain an hystidine as linker for the porphirine donor and it is covalently bound by its N-term portion to a loop system incorporating the acceptor, namely a substituted quinone. A simulation of the designed peptide using molecular modeling code GROMOS has been carried out, to gain confidence into the theoretical alphaelical structure. The peptide was synthesized by SPPS metodology, using *Fastmoc*, HBTU protocol. The product was evaluated by Mass Spectrometry and high-performance liquid chromatography (HPLC). Subsequently, it was purified to be used in NMR and CD experiments, to investigate the structure of this element of the whole maquette.

Keywords: electron transfer, membrane, biological systems.

O23

Two Photosystem II-Based Biosensors for Detection of Photosynthetic Herbicides

M. Koblízek[1,2], J. Masojídek[2], J. Komenda[2], T. Kucera[3], A. K. Mattoo[4], M. T. Giardi[5] and R. Pilloton[6]

[1]Dept. of Plant Physiology, University of South Bohemia, 370 05 Ceské Budejovice, Czech Republic
[2]Institute of Microbiology, Academy of Sciences, 37981 Trebon, Czech Republic
[3]Dept. of Biochemistry, Charles University, 12840 Prague, Czech Republic
[4]Vegetable Laboratory, USDA/ARS/BARC, Building 010A, Beltsville, MD 20705, USA
[5]IBEV-CNR, Via Salaria Km 29.3, 00016 Monterotondo Scalo, Italy
[6]New Technologies Dept., ENEA, Casaccia, Via Anguillarese 301, 00060 Rome, Italy

We have developed two kinds of electrochemical biosensors for the detection of residual triazine-, urea- and phenolic-type herbicides, using isolated photosystem II (PS II) particles from the thermophilic cyanobacterium, Synechococcus elongatus, as biosensing elements. The herbicide detection was based on the fact, that in the presence of artificial electron acceptors, the light-induced electron transfer through isolated PSII particles is accompanied by the release of oxygen, which is inhibited by the herbicide in a concentration-dependent manner. In the first biosensor, the PSII particles were entrapped between the dialysis and Teflon membrane of the Clark oxygen electrode. The decrease of their oxygen evolution was used as a measure of herbicide concentration. In the second biosensor the rate of electron acceptor reduction was measured using a screen-printed electrode. The PSII particles were immobilised on the working electrode using BSA and glutaraldehyde. The combination of highly active PSII particles with the flow system resulted in re-usable herbicide biosensors with good stability (half-life of first biosensor was about 35 h work at 25°C) and high sensitivity (detection limit for diuron was 5×10^{-10} M).

Keywords: photosynthesis, biosensor, herbicides, flow.

e-mail: Pilloton@mclink.it

O24

Effect of Heavy Metals on the Structure and Function of Photosystem II: Potential and Prospects for Use as Bioindicator

Jirí Masojídek[1], Jan Malý[1,2], Sabrina Alessandrelli[3], Michal Koblížek [1,4], Marco Rizzuto[3], Bettina Geiken[3], Jirí Kopecký[1], Josef Komenda[1], Ondrej Prašil[1] and Maria Teresa Giardi[3]

1Institute of Microbiology, Academy of Sciences, 37981 Trebon, Czech Republic
2Dept. of Biology, University of J.E. Purkyne, 400 96 Ústí n. Labem, Czech Republic
3IBEV-CNR, Via Salaria Km 29.3, 00016 Monterotondo Scalo, Italy
4Dept. of Plant Physiology, University of South Bohemia, 370 05 Ceské Budejovice, Czech Republic

Heavy metals (HMs) exert multiple inhibitory effects on photosynthesis at different structural and metabolic levels. Interaction of HMs with the functional SH-groups was generally proposed as the mechanism for several inhibitory reactions. It has been suggested that the Calvin cycle reactions are more likely to be the primary targets of the toxic effects of HMs. Subsequently, the reduced demand for ATP and NADPH upon the Calvin cycle causes a down-regulation of both photosystem II photochemistry and of linear electron transport. A strong influence of cadmium at the first stage of plant treatment on D1 protein turnover has been observed. Pulse-chase experiments in vivo followed by the separation of thylakoids into grana and stroma exposed regions indicated that the synthesis, degradation and assembly of the D1 protein can be greatly affected by HMs. Modulation of D1 turnover under stress is a commonly occurring process. Monitoring the effect of HMs (Cd, Cu, Zn) and As in algal cells by more advanced biochemical and biophysical techniques (fluorescence microscopy, fluorescence induction using double-modulation fluorometry, thermoluminescence) was done in order to obtain a more detailed information on the mechanism how HMs affect the photosynthetic apparatus. The complex scheme of the possible inhibition mechanism of the photosynthetic apparatus under the HM stress was proposed by Malý (1998).
The effect of HMs on the function of PSII was employed in design and construction of biosensors based on isolated thylakoids and PSII particles as well as intact cell of algae. The use of biosensors open a possibility for monitoring environmental pollution of aquatic and terrestrial ecosystem by HMs. When compared with classical analytical methods (HPLC, ELISA etc.), the biosensors can provide more precious information about real biological effect of pollutants since phytotoxicity is determined from the measurement of electron transport activity, photocurrent or photosynthetic oxygen evolution.

Keywords: D1 turnover; biosensor; heavy metal; photosystem II; fluorescence; thermoluminescence

Abbreviations: HM, heavy metal; PSII, photosystem II

O25

Tannery process regulation with on line chromium (III) monitoring

Massimo Pizzichini[1], Mauro Spadoni[1], Rosalba Montani[2].

[1]ENEA, C.R. Casaccia, Via Anguillarese 301, 00060 Roma
[2]INTEAM s.r.l., Via Chiana 48, 00198 Roma

A special sensor for chromium (III) on line monitoring into the tanning reactor was studied and developed for a practical use in tannery industry.
The sensor was carried out combining different apparatus as ultrafiltration unit for cleaning the tanning solution, flow injection system to increase the monitoring range, and a photometer detector with wavelength set between 440 and 460 nm.
The chromium sensor is hydraulically connected with a tanning big drum, through a recycling loop in which are inserted some filtration apparatus.
The sensor flow cell is fed with water solution at a flow rate of 5 ml/min that assures a suitable sample carrying out and a continuous cleaning of the system.
A sample loop of about 30 ml provide a suitable photometric response, with characteristic peaks having an area proportional to chromium concentration.
The sensor shows a satisfactory linear response in the 2-20 g/l chromium concentrations range, with an accuracy of 5 %.
The single determination take place in 30-50 seconds and the measure is not disturbed by organic chemicals present in the tanning medium. The peaks area are integrated in order to have directly the chromium concentration in g/l, as requested by the tannery operators.

Keywords: chromium(III), automation and process control, tannery.

O26

Screen-Printed Electrodes for the Detection of Heavy Metals

Ilaria Palchetti, Luciana Lepore, Marco Mascini

Dipartimento di Sanità Pubblica Epidemiologia e Chimica Analitica Ambientale, Università degli Studi di Firenze, Via G. Capponi 9, 50121, Firenze, Italia
http://www.igiene.unifi.it/chimica/sensori

The use of disposable screen-printed electrodes for stripping analysis is described. The graphite surface of the working electrode can be used as substrate for plating a thin mercury film, which allows the electrochemical preconcentration of heavy metals. Detection limits around ppb level were obtained for different metals (Pb(II), Cd(II), Cu(II)). Moreover chemically modified screen-printed electrodes were developed in order to have mercury-free devices to accumulate heavy metals. Different substances (ion-exchangers and ligands) were tested.

Keywords: screen printed electrodes, stripping analysis, heavy metals

O27

Organic and biological molecules photoionization for the fabrication of bioelectronic devices

P. Morales[1], L. Giulietti[1], L. Mosiello[1], R. Pilloton[1], F. Bordoni[2], G. DeGasperis[2], S. Sperandii[2], S. Santucci[3], E. Di Fabrizio[4]

[1]ENEA, Centro della Casaccia
[2]Facolta' di Ingegneria, Universita' di L'Aquila
[3]Dipartimento di Fisica, Universita' di L'Aquila.
[4]Istituto di Elettronica dello Stato Solido del CNR, Roma

This work aims to the development of a fabrication technique for miniaturized bioelectronic devices that is fully compatible with the techniques used in conventional solid state microelectronics. The implications of the method span from clinical use biosensors to DNA sequencing to truly molecular electronics.
Our technique is based on laser pulse vaporization of organic and biological molecules in a high vacuum environment and on the subsequent multiple photon ionization of the vapour phase molecules by a second selectively tuned laser pulse The ions produced can then be directed to the deposition substrate by means of electric fields; high resolution patterning can be achieved by tghe insertion of microlithographic masks on the ions trajectory or by localizing the field using thin moveable tips such as those of Scanning Probe Microscopes.
In this work we checked the biological functioning of biomolecules deposited by this method, and we studied the fragmentation of organic molecules after their ionization in vapour phase. We also studied the adhesion of the molecular layers to the substrate and, by means of suitable patterns, we determined thickness (few molecular layers) and uniformity of the layers by AFM microscopy. Depositions have been achieved both through masks, with resolutions of the order of 100 nm, and by electric fields localized on a few microns range; in this latter case we also tried moving the field spatially to pattern the deposition at low resolution. We also tried the deposition of metallic layers by the same technique in order to attempt the fabrication of submicroscopic conductors and electrodes.
Finally, we attempted the fabrication of a prototype of a 10 micron large glucose biosensor, of which we measured the qualitative response to glucose injections.

Keywords: photoionization, miniaturization, bioelectronic devices.

O28

Screen Printing of Enzyme or Selective Polymer Inks for Chemical Sensors and Biosensors Mass Production

S. Marino, G. Minervini, M.R. Montereali, R. Pilloton, C. Cremisini

CR-Enea Casaccia, SP008-Via Anguillarese 301 - 00060 Rome Italy

Screen printing technology seems to be one of the most promising technologies allowing biosensors, the analytical devices based on the use of biological mediators, to be largely on the market in the next future because of the chance of mass, low price production and high reproducibility. Amperometric or conductivity electrodes were printed on ceramic or polymeric substrates and coupled with biomediators or molecular imprinted polymers with the aim to obtain biological or chemical sensors. The procedures for obtaining porous alumina based ceramic devices allowing improved performances were studied. Some examples of enzyme based electrochemical biosensors useful in the environmental analysis are reported.

Keywords: screen printing, enzymes, biosensors, molecular imprinting.

e-mail: Pilloton@mclink.it

O29

Simple and fast determination of Lactose and Lactulose in row and UHT milk using differential pH-technique

Silvia De Vita[1], Nicoletta Luisetto[1], Costante Pinelli[2], Evaristo Dalla Turca[2]

[1]EurochemTecnologie Analitiche, I-00040 Via Pontina Km.34, Ardea, Rome
[2]Parmalat S.p.A., I-43044 Via Milano 1, Collecchio, Parma

A simple and fast method for the determination of Lactose and Lactulose has been evaluated. The method is based on the correlation of pH differences, measured with a glass electrode, generated by H^+ or OH^- produced in the medium by enzymatic reaction. The result, obtained determining Lactose and Lactulose were compared with HPLC. The procedure based on the differential pH-measurements has been found to give results comparable to that of official method. The precision obtained using the two procedures was not significantly different.
The proposed method is resulted simple and fast (less then 4 minutes) , particularly for the lactulose evaluation considering no sample pre-treatment is required for measurements which are also automatically recorded. We suggest using the proposed method, based on differential pH-technique, for routine determination of Lactose and Lactulose in raw and UHT process treatment.

Keywords: milk, lactose, lactulose, differential pH technique.

P01

Ellipsometric and surface plasmon resonance effects on LB films of organic materials in controlled atmosphere

[1]R. Rella, [1]P. Siciliano, [2]L. Valli, [2]L. Vasanelli

[1] Istituto per lo studio di nuovi Materiali per l'Elettronica IME – CNR via Arnesano, 73100 Lecce, Italy
[2] Dipartimento di Scienza dei materiali, Universita' di Lecce, Via per Arnesano 73100 Lecce, Italy

Langmuir-Blodgett (LB) films of Cu(II) tetrakis-(3,3-dimethyl-1-butoxicarbonyl)phthalocyanine have been studied as regard their optical property. In particular electronic spectra in the UV-VIS spectral range and the optical constant of the LB multilayers have been carried out. In order to use these LB films as optical gas sensors, ellipsometric and surface plasmon resonance (SPR) measurements were carried out in controlled atmosphere. SPR measurements were conducted by using a system based on the Kretschmann configuration. LB monolayers of Cu(dmbc)Pc deposited as selective layer on a metal surface show changes of the reflectance on exposure to nitrogen dioxide mixed with dry air in low concentration. Moreover, ellipsometric measurements carried out on LB multilayers of Cu(dmbc)Pc deposited onto silicon substrates, have shown variation at a fixed wavelength in the thickness and complex refractive index when are exposed to different concentrations of toluene and tetrachloroethene.

Keywords: Lamgmuir-Blodgett; optical properties, chemo-optical sensors

P02

Immobilization and adsorption of idrolase onto aerogel matrices: a preliminary study

M. Casarci[1], O. Li Rosi[1], C. Neri[1], L. Gardossi[2], C.Erbert[2], L. De Martin[2]

[1] ENEA, C.R.Casaccia Via Anguillarese 301, Rome, Italy

[2] Dipartimento di Scienze Farmaceutiche, P.le Europa, 1, Trieste, Italy

Silica alcogels were made with tetraethyl orthosilicate (TEOS, $Si(OCH_2CH_3)_4$) as precursor in basic media.
The dryed silica aerogels have unique properties such as high porosity, large surface area, and low density, depending on water - TEOS ratio.
The aim of this study is to check the applicability of the porous silica aerogel matrix, for the immobilization and adsorption of *Pennicillin G amidase* enzyme (PGA). Preliminary results are shown.

Keywords: silica aerogel, biocatalysis, *Pennicillin G amidase*, biotransformation.

P03

Feasibility of an Optical Biosensor Based on Porous Silicon

G. Di Francia[1], L. Quercia[1], S. La Ferrara[1], S. Manzo[1], S. Chiavarini[2], F. Cerullo[3], F. De Filippo[3], V. La Ferrara[3], P. Maddalena[3], R. Vitiello[3]

[1]CR ENEA Portici, Localita' Granatello, 80055 Portici, Italy
[2]CR ENEA Casaccia, Via Anguillarese, 00060 Roma, Italy
[3]INFM and Dipartimento di Scienze Fisiche, Monte Sant'Angelo, 80100 Napoli, Italy

Porous Silicon thin films, obtained by p-type silicon, have been used for developing an optical interferometric biosensor. Its surface has been modified using a bromine oxidation to prepare porous silicon for DNA attachment. Optical characterization has been made either for as prepared samples and for oxidized ones. Modelling shows that large reflectance variations can be expected when such a treated PS surface is exposed to even small quantities of DNA.

Keywords: porous silicon, biosensor

P04

Dipstick immunoassay format for atrazine and terbuthylazine analysis in water samples

L. Mosiello[1], C. Cremisini[1], L. Segre[1], S. Chiavarini[1], M. Spanò[1], T. Kimmel[2], A. J. Bäumner[2] and R. D. Schmid[2]

[1]ENEA, Department of the Environment, C.R. Casaccia - Via Anguillarese 301, 00060 Rome, Italy.
[2]Institute for Technical Biochemistry, University of Stuttgart Allmandring 31, D-70569 Stuttgart, Germany

A new dipstick immunoassay format for terbuthylazine determination, based on the use of polystyrol strips as antibody coating support, is described. Terbuthylazine calibration curve show linear measuring range between 1.2-10 mg/L and a practical detection limit of 1.2 mg/L. Real water samples were analysed and results correlate well with those from gas chromatography (GC/MS) and ELISA. No false negative results were evidenced.A dipstick immunoassay format for atrazine determination was also used for the analyses of the same samples. Some false positive results were obtained, probably due to cross-reactivity of the atrazine monoclonal antibody for terbuthylazine.At this developmental stage, these dipsticks can be very useful as qualitative/semiquantitative "field test" for identifying "positive" samples, reducing the number of samples to be reanalysed in laboratory according to analytical standard methods (GC). Further improvements are possible in order to optimise the whole system on the strictly analytical aspects.

Keywords: atrazine; terbuthylazine; dipstick immunoassay; enzyme immunoassay; environmental monitoring; water samples.

P05

Evaluation of PSII-based biosensor for herbicide analysis in river by comparison with electrophoretic and chromatography techniques

M Rizzuto[1], R Pilloton[2], C Desiderio[1], C Polcaro[3], MT Giardi[4]

[1]ICN, [3]IC, [4]IBEV-Area della Ricerca di Roma, 00016 Monterotondo Scalo, Italy
[2]Enea C.R. Casaccia Via Anguillarese 301, 00060 Rome, Italy

Photosystem II is the enzimatic complex supporting photosynthetic electron transport, splitting water and producing ATP and NADPH in photosynthetic organisms. For its inherent feature Photosystem II (PSII) is extremely suitable for the realization of biosensors. Upon illumination PSII runs electrons and fluorescens, reactions that are inhibited in the presence of photosynthetic herbicide. One of the advantages is the simplicity of the biological transduction which can be monitored directly without requiring other markers or transducer molecules. Another advantage is that PSII is extremely susceptible and selective to some agents; these agents are widely used in agriculture as herbicides. Phenylurea, triazine, diazine and phenolic derivatives represent economically very important compounds since they are used in chemical, pharmaceutical and agricultural industry. They still represent about 50% of all herbicides used at the present in agriculture, with a world use of many thousands of tons. Recent work showed the possibility to isolate quite stable PSII particles and building a flow cell provided of Clark's electrode a very high sensitivity (in the nanomolar range for the herbicide) of the developed biosensor was obtained (1).
In the present work we tried to test the practical application of the biosensor for monitoring under real operational conditions.
In the first step we analyzed the modification of PSII activities in various waters. We observed that PS II activity is stimulated by 10-15% in normal water and the nature of stimulation was partially attributed to the presence of bivalent ions. The bivalent ions cause a different staking in the membrane with consequent change of electron transfer capacity. A comparison of the biosensor analytical response with capillary electrophoresis and HPLC techniques was performed.

Keywords: biosensor, PSII, herbicide determination.

P06

Effects of gamma-radiation on Photosystem II activities for the realisation of biosensors

G. Angelini[1], E. Pace[1], D. Esposito[1], M. Rizzuto[1] and M.T. Giardi[2]

[1]ICN,
[2]IBEV-CNR. Via Salaria km 29,3 - 00016 Monterotondo Scalo, Italy

Photosystem II is an enzimatic chlorophyll-protein complex which, in higher plants and photosynthetic organisms, catalyses primary charge separation and is responsible for splitting water to form molecular oxygen, thus supporting electron transfer and the photosynthetic process. The Photosystem II as a biosensor has several advantages.
The most important is the simple nature of the biological transduction which is directly usable without resorting to makers or competitors. The decrease of the oxygen-evolution, the electron transfer, and the fluorescence can be easily monitored using Clark's electrodes, potenziometric and optical systems, respectively. Given the sensitivity of the PSII complex to several pollutants and stress conditions, it has the potential for a wide variety of applications.
The aim of the present work was to study the potential of Photosystem II for use as a biosensor to detect ionizing radiation. Atomic physics reveals that the passage of radiation through matter is characterised by a transfer of energy to the target material. Exposure of biological material to ionising radiation leads to a loss of function due to the destruction of critical structures. The larger the structure, the more likely it will be hit. Because the energy deposition is so large, function is completely destroyed by a single hit. The only activity remaining after radiation exposure is a result of units which have escaped ionisation and are fully active. Therefore, the level of PSII activity decline in oxygen-evolution, fluorescence or electron transfer can be directly correlated to the dose of radiation.
We studied the effects of γ -ray exposure in a ^{60}Co-cell of 8 Mrad on Photosystem II activities. We observed that at –20°C, the activity of isolated PSII particles is inhibited to 90% after 10h g -ray exposure; in 1h the functionality is decreased by about 6-10%, corresponding to an absorbed energy of 7000 Gy (Joule/Kg). An application of the biosensor could be the measurement of the space radiation. Galactic cosmic radiation consists of about 83% of protons with energies between 10-1000 MeV, 16% helium nucleus and 1% electrons. Despite the high energies involved, the number of these particles is very reduced to a flux of 0.01 erg. cm^{-2}.sec^{-1}. Under such a condition, the biosensor tested in our laboratory would not be sensitive enough since it would adsorb an energy of 7000 Gy only after 800 days of exposition. However, solar particle radiation,

contains energetic particles emitted more copiously during magnetic disturbances. The energy released during a sun flare is about 10^{32} erg and the flux can be a thousand times higher than galactic cosmic radiation. Under these conditions we calculated that our actual biosensor system could absorb energy enough to inhibit its functionality in 1-2 days.
We studied the effects of radiation on various organisms coupling the biomediator to a PEA fluorescence transduction system. It was found that various organisms respond to radiation in a different way and the sensitivity to radiation was correlated to the lipid composition. We designed a semiautomatic system to renew the inhibited biomediator.
In comparison to the present non-traditional system for measuring ionising radiation with the current methods (counters and visual detectors), this device provides many advantages: i) miniature size, ii) modulated sensitivity depending on the biological preparation; iii) measurements on line; iv) low expense; v) operative for long-term experiments. Moreover, this system directly measures the radiation effect on living photosynthetic organisms which are expected to be used as an oxygen-producing system on shuttles.

Keywords: gamma radiation, PSII, biosensor.

P07

A Small and Inexpensive Biosensor based Device for On Line Evaluation of Microalgae Metabolism Inhibition

A. Masci, S. Galluppi, C. Micheli and R.Pilloton

ENEA CR. Casaccia C.P. 2400 / 00100 Roma, Italy

A biosensor based device was assembled with the aim to investigate on algal primary production as crucial process in the life dynamic and health state of aquatic ecosystems. The new device is based on a fast and not expensive "on line" micro-electrode system for the determination of the inhibition of algal metabolism. Oxygen evolution induced from the light was determined using a small number of living algae entrapped in an agarose gel matrix and deposited on a screen printed microelectrode. The photosinthetic biosensor was placed in a flow cell obtained by carving a green light emitting diode (LED). The micro-electrode was cut from a PVC sheet on which graphite (Pt) and silver based pastes were deposited on both sides by screen printing. As a result a photosynthetic biosensor working in sea water without artificial electron acceptor or other activators added in solution was obtained. Additionally photosynthetic parameters obtained by different methods such as Clark-type oxygen electrode and *Pump and probe* technique were compared with the new method.

Keywords: algae, photosybthesis, inhibition, biosensor, device, screen printing

e-mail: Pilloton@mclink.it

P08

Investigation of the effect of temperature gradients on theresponse of a glucose biosensor

M. Santucci[1,2], M. Portaccio[1,2], M.S. Mohy Eldin[1], S.Rossi[1], U. Bencivenga[1], F.S. Gaeta[1] and D.G. Mita[1,2]

[1]Istituto Internazionale di Genetica e Biofisica, CNR – Via G. Marconi, 10 - 80125 Napoli, Italy

[2]Dipartimento di Fisiologia Umana e Funzioni Biologiche Integrate "F. Bottazzi", Facoltà di Medicina e Chirurgia, II Università di Napoli, Via S.M. di Costantinopoli, 16 – 80138 Napoli, Italy

The effects of a temperature gradient applied across the catalytic membrane of a glucose biosensor have been studied with reference to its characteristic parameters. Dynamic response, steady-state response and response time have been investigated as a function of glucose concentration and of the applied temperature difference. The results obtained under non-isothermal conditions, compared with those obtained under comparable isothermal conditions, evidenced an increase in the dynamic and steady-state responses as well as in the sensitivity and a decrease of the response times. These results suggest the opportunity of designing a new class of biosensors.

Keywords: non-isothermal biosensors, glucose determination, glucose oxidase, grafted membranes, biocatalytic membranes, biosensors.

P09

Superoxide and nitric oxide radicals as modulating agents of enzymatic sensor responses

L.Campanella, L. Persi, M.Tomassetti

University of Rome "La Sapienza" Chemistry Departement.
P.le Aldo Moro, 5-00185 Rome (Italy)

A study was made of the modulating action on the enzymatic activity of the enzymes tyrosinase and superoxide dismutase by two respectively interfering radicals - the superoxide and the nitric oxide radical. This was done using a tyrosinase and superoxide dismutase biosensor, respectively, the response of which to the respective substrates had been recorded in the presence and absence of interfering agent In both cases. The concentration of the interfering radicals may be determined by constructing previously suitable calibration curves.

Keywords: superoxide, nitric oxide, radicals, detection, biosensors.

P10

Silicon microcantilever based biochemical surface stress sensors

R. Raiteri[1,2], S. Martinoia[1], M. Grattarola[1], H.-J. Butt[2]

[1]Università di Genova - DIBE - Dipartimento Ingegneria Biofisica ed Elettronica
[2]Institute for Physical Chemistry, Johannes Gutenberg University of Mainz, Germany

We show that the bending-plate method with microfabricated cantilevers can be used to transduce the binding of a biological substance to a receptor into a signal. This could be the basis for a new class of biosensors. To demonstrate the feasibility of the method we coated cantilevers with the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D). Then we measured the cantilever deflection while continuously rinsing with a solution containing the monoclonal antibody. In this way, we monitored changes in the surface stress of the cantilever due to the binding of the antibody.

Keywords: atomic force microscopy, biosensor, immunosensor, bending-plate, microfabrication

P11

Optical characterization of a new dye covalently bound on controlled pore glasses potentially suitable as optical transducer for Hg(II)

A. Flamini,[1] F. Baldini[2], A. Falai[2]

[1] Istituto di Chimica dei Materiali, ICMAT-CNR
Via Salaria, CP10, 00016 Monterotondo Stazione, Roma, Italy
[2] Istituto di Ricerca sulle Onde Elettromagnetiche "Nello Carrara", IROE- CNR Via Panciatichi 64, 50127, Firenze, Italy

The current paper describes a novel absorption-based optode for mercury (II) detection. The dye 2-(5-amino-3,4-dicyano-2*H*-pyrrol-2-ylidene)-1,1,2-tricyanoethanide (L', C_5N_3-$C_4N(CN)_2$-NH_{2-}), which is a specific indicator for Hg(II) undergoing a colour change from violet to blue on the formation of the corresponding monochelated [HgL] complex, was covalently bound on a suitable matrix and its optical characterization as optode was performed in the presence of mercury acetate [$Hg(AcO)_2$] aqueous solutions.

Keywords: optical fibre sensors, mercury, absorption, optode

P12

Polymeric Membranes For Humidity Sensors

A. Furlani[1], M. V. Russo[1], P. Altamura[1], I. Fratoddi[1], and C. Caliendo[2]

[1]Dipartimento di Chimica -Università "La Sapienza"- P.le Aldo Moro 5 - 00185 Roma, Italy
[2]Istituto di Acustica "O. M. Corbino" - Area di Ricerca di Tor Vergata - Via del Fosso del Cavaliere,100 - 00133 Roma, Italy

Electrical and SAW sensors have been performed by spinning p - conjugated polymer membranes on appropriate substrates. Polymers from monosubstituted acetylenes
H-C° C-R (R=C_6H_5,; $C_{13}H_8OH$; CH_2OH ; $CH_2N(CH_3)_2$) and polymers of the general formula [-$M(PBu_3)_2$-C° C-C_6H_4-C_6H_4-C° C-]$_n$ (M = Pt, Pd) were syntesized and investigated. These polymers can be easily deposited to give homogeneous membranes sensitive to R.H. variations. The responses of different devices are compared. Their behaviour is dependent on the R pendant group of the polymeric chains, on the doping agents and on the chain metal in the [-$M(PBu_3)_2$-C° C-C_6H_4-C_6H_4-C° C-]$_n$ polymers. The sensors exhibit different sensitivities in different R.H. ranges. An appropriate choice of sensors can therefore better cover the complete R.H. range from 0 to 90%.

Keywords: polyacetylenes-metal containing polymers-electrical and saw sensors

P13

Monitoring water quality in aquaculture with a new sensor for microorganisms and toxicity

AnnaRosa Sprocati[1] Vincenzo Capuano[1] Giovanni Antonini[2] Pierdaniela Valenti[3] Alessandra Aiello[4]

[1] ENEA-Dip.Innovazione Div. Biotecnologie
[2] Dip. Biologia di Base e Applicata - Università dell'Aquila
[3] Istituto di Microbiologia II° Università di Napoli
[4] MICROBO srl

A correct risk assessment in aquaculture activities must take into account both inflowing as outflowing waters, either in order to control risk factors for fish populations under stressful conditions or in order to avoid negative environmental impacts of the aquaculture plant. The development of innovative methods of control can positively contribute to a correct management of aquaculture activities. In the present work, sensor prototypes were installed on a mobile laboratory and utilised for monitoring faecal contamination and toxicity in a brackish aquaculture plant, situated on the Orbetello Lagoon, Italy. The final aim of the work was to verify the possibility to control the feacal contamination in real time, with reduced analysis costs, without analytical discontinuity, with automated data acquisition and employing non-specialised personnel. Thus the objectives were to conduct a methodological comparison between the sensor and the classical microbiological methods in brackish waters and to verify the efficiency of the prototypes over a long-time working period. The work has been split up into 5 campaigns during different seasons. Three different sampling points within the plant have been selected for which the following parameters were monitored: *(i)* faecal contamination index, *(ii)* nutrient content, *(iii)* pH and *(iv)* temperature. Sampling frequency was 2X a day during each campaign The sensor, patented by Microbo srl, is able to automatically measure the concentration of either total coliforms or faecal coliforms or faecal streptococci. The principle is based on the detection of bacterial metabolism, instead of replication (classical counting methods), through a specifically designed medium, added to the sample, in which products with oxido-reductive activity are contained and are metabolised by the microorganisms. The redox variation is converted into a change of electrical intensity or into a voltage change and compared to a reference electrode. The time required for the redox reaction is inversely proportional to the number of microorganisms present, varying e.g. for total coliforms, from 45 minutes to 12 hours, in case of sterility. Each prototype can process one sample at a time. The sensor can also be used for a toxicological

test, which is based on the absence or reduction of microbial metabolism (a mutant strain of *E. coli* pva 45) in the presence of toxic substances. The lower range of sensibility is at a concentration of 10^{-4}M. Validation has been done on phenol.

Final results showed that the potentiality of this sensor is interesting and that the biological method is also very reliable for brackish waters. Nevertheless an improvement of the machinery is required to tolerate long-time working periods without specialised personnel and to carry out multiple samples at the same time. Indeed when the microbial concentration was lower than $10^{4,}$ only 39% of the foreseen analysis have been done within 24 hours, which is the time required by classical methods for T.C. For concentrations higher than $10^{4,}$ the total time of analysis has been shorter than classical methods. The toxicological test showed a light toxicity of the inflowing waters.

This work has been supported by the Ministero della Marina Mercantile in the Fourth National Plan for Agriculture and Fishery.

Keywords: environmental biotechnology

P14

Construction and application of highly selective sensors and biosensors using non-conducting electropolymerized films

A. Curulli[1], and G. Palleschi[2]

[1]Centro CNR di studio per L'Elettrochimica e la Chimica Fisica delle Interfasi Via del CastroLaurenziano 7 000161 Roma, Italy
[2]Dipartimento di Scienze e Tecnologie Chimiche Università di Roma Tor Vergata Via della Ricerca Scientifica 00133 Roma, Italy

Different kind of macrosensors have been assembled using several non-conducting electropolymerized films. Different techniques of film electropolymerization such as cyclic voltammetry and chronoamperometry are compared. Film function, including the prevention of interferences and fouling problems and immobilization of the enzymes are described and optimised. We have immobilized oxidase [1] and dehydrogenase enzymes [2] onto electropolymerized films with different techniques, depending in the nature of the enzyme. We have, also, developed an interference-free highly selective sensor for the detection of NO in biological media [3], using non-conducting electrosynthesized films

Keywords: electropolymerization, sensors, biosensors.

P15

Cloning of novel seven-transmembranes-domain receptors from rat olfactory neuroepithelium

Alessandra Crisà, Federica Forbici, Maria Teresa Mancuso and Giacomo A.F. D'Agostaro.

Dipartimento Ambiente, ENEA, Casaccia, Roma.

The olfactory system of vertebrates is able to translate olfactory stimuli into complex sensory information. The olfactory transduction is initiated by the binding of an odorant ligand to a protein receptor on the apical surface of olfactory sensory neurons. The olfactory receptors are 7-transmembrane-domain proteins belonging to a large multigene family. The rat OR gene family has been estimated to include approximately 500-1000 genes.
In initial experiments, we designed a series of degenerate oligonucleotides that could anneal to conserved regions of 96 members of the OR gene family. The synthetic oligonucleotides were used in PCR reactions to amplify homologous sequences in cDNA synthesized by reverse transcription of polyA$^+$ RNA purified from rat olfactory neuroepithelium. The amplification products were within the size range expected for this family of receptors. The PCR products were subsequently inserted in a plasmid vector and individual clones were isolated. Sequence analysis of 48 random clones revealed that all were new members of the OR family and were distributed broadly across a similarity dendrogram.
Two amplification product showing the highest sequence divergence were used as molecular probes to screen a cDNA library constructed from rat olfactory neuroepithelium polyA$^+$ RNA and cloned in a phage vector. Three full-length cDNA clones were isolated by screening 3.6x 10^5 recombinant phages. Sequence analysis of the cloned cDNAs showed that each clone is a new member of the OR family. In situ hybridization experiments indicate that these OR genes are espressed selectively in the sensory neurons of the rat olfactory neuroepithelium.

Keywords: olfactory system, receptors.

PARTECIPANTI

Manuela Adami
A.Aiello
Sabrina Alessandrelli
Giancarlo Angelini
G.Antonini
Loris Arbizzani
Luciano Baiocco
Francesco Baldini
Eugenio Benvenuto
Rita Bernardo
Paola Bolle
Cristiana Bongiovanni
Claudio Botrè
Francesco Botrè
Carla Brofferio
Marzia Bruno
Franco Campagnola
Luigi Campanella
Stefano Canese
Silvia Canofeni
Cristina Cantale
S. Capone
Paolo Capocecera
Vincenzo Capuano
Giuseppe Caputo
Giovanni Carducci
Maurizio Casarci
Stefania Cesa
Lucia Ceresa
Francisco J. Comendador
Dario Compagnone
Marcelo Enrique Conti
Carlo Cremisini
Antonella Curulli
Giacomo D'Agostaro
Flavia Dalla Riva
Laura Del Signore
Claudia Desiderio
Silvia De Vita
Valeria Di Carlo
Girolamo Di Francia
Giorgio Di Marco
Daniele D'Ottavi
Claudio Domenici
Renato Donati
M. Epifani
Dania Esposito
Maria Grazia Evandri
Claudio Fabiani
Alida Falai
Gabriele Favero
Tommaso Ferri
Alberto Flamini
Rosella Franconi
Anita Furlani
Simona Galluppi
MariaTeresa Giardi
Giovanni Giuliano
Michal Koblizek
V. La Ferrara
Gina Isoldi
Chiara Leonardi
Ornella Li rosi
Sonia Manzo
Stefano Marino
Giuliano Martinelli
Amedeo Masci
Marco Mascini
Jiri Masojidek
Franco Mazzei
Giustino Mele
Fernando Messeri
Carla Micheli
Laura Micheli
Carmelo Mignosa
Giovanni Minervini
Rosalba Montani
MariaRita Montereali
Piero Morales
Danila Moscone
Lucia Mosiello
Luigi Nardi
N. Nedeljkovic
Claudia Neri
Emanuela Pace
Ilaria Palchetti
Giuseppe Palleschi
Paolo Pelosi
Michele Penza
Krishna Persaud
Ferdinando Perrotta
Roberto Pilloton
Luisa Pirozzi
Massimo Pizzichini
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Chiara Polcaro
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R.Raiteri
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Marco Rizzuto
Aldo Roda
L. Quercia
Riccardo Rizzi
Luca Romanelli
Giovanna Rosso
Maria Vittoria Russo
MariaPia Sammartino
Paolo Santaroni
Massimo Santucci
Roberto Santucci
Luciano Saso
Giorgio Sberveglieri
Giancarlo Scibona
Piero Siciliano
Bruno Silvestrini
Maria Sperandei
AnnaRosa Sprocati
Mauro Tomassetti
Sara Tombelli
Luigi Tommasino
Marcello Trevisani
Paolo Tucci
P.D. Valenti
L.Vasanelli
Giuseppe Vasapollo
Simona Villari
Giulia Volpe
Richard A. Zuccolo

INDICE DEGLI AUTORI

PAROLE CHIAVE

II WORKSHOP
SENSORI CHIMICI e BIOSENSORI

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HTTP://WWW.UNIROMA1.IT/BIOPROBE/WORKSHOP.HTM

Roberto Pilloton
ENEA, INN-BIOAG-BIMO, SP008
Via Anguillarese 301 - 00060 – Roma
E-mail: pilloton@mclink.it
http://inn7201.casaccia.enea.it

Franco Mazzei
Universita' "La Sapienza", DSCTSBA,
P.le Aldo Moro 5, 00185 Roma
E-mail: mazzei@uniroma1.it
http://www.uniroma1.it/bioprobe

M.T. Giardi - G. Angelini
CNR-IBEV-ICN,
Via Salaria Km 29.3,
00016 Monterotondo Scalo, Roma

Segreteria Organizzativa
MariaRita Montereali
Tel.: 06-30483294
Segr.Tel.: 06-30483814
Fax: 06-30483327
E.mail: Biosensori@casaccia.enea.it

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PALL Gelman Laboratory

Divisione della Pall Italia s.r.l.
Via G. Murat, 84 - 20159 MILANO
Tel.: 02-69.00.61.09 - Fax : 02-69.00.61.10