BOOK OF ABSTRACTS

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- Valentina Lanzone, vlanzone@unite.it

PLENARY LECTURES

PL1

15 YEARS OF PORPHYRINS-BASED GAS SENSORS AT ROME TOR-VERGATA

Corrado Di Natale

Department of Electronic Engineering, University of Rome Tor Vergata
dinatale@uniroma2.it

Sensors are devices that convert the concentration of chemical compounds into a measurable quantity. Current technological development privileges electric quantities for which the measurement, the storage, the elaboration, and the transmission of information is performed with the maximum efficiency. In practice, sensors are electronic devices whose parameters depend on some external quantity of whatever nature. In particular, they are devices that from the electronic point of view are resistors, capacitors, or even diodes or transistors, whose electrical parameters depend on the chemical composition of the environment at which they are in contact.

In case of chemical sensors, since electronics properties of materials are only in some cases influenced by the ambiental chemistry, an ancillary phenomenon is necessary to connect the electronic properties with the chemical interactions. As a consequence, chemical sensors are formed by two components: the chemically interactive material and the basic transducer. Several choices for chemical interactive materials are available. In particular, molecular materials are appealing because of the relative facility to synthesize molecular units endowed with different chemical functionalities. Among them, porphyrins are excellent example of chemically interactive materials. The rich chemistry of porphyrins results in a large variety of interaction mechanisms that can be exploited for chemical sensing. While the role of metal is considered of primary importance to determine the sensitivity and selectivity properties of the macrocycle, by coordination of the volatile molecule, so mimicking the biological functions of these compounds, hydrogen bond, polarization, and polar interactions may contemporaneously be present and cooperate in the total guest molecule binding. Besides to drive the chemical sensitivity, these interactions offer also the possibility to drive self-assembled molecular aggregations with distinct chemical properties.

The interactions between guest molecules and solid-state layer of porphyrins can modify the physical properties of the porphyrins layer. Properties such as conductivity, work function, mass and optical absorbance are among those that can be readily converted into electric signals by suitable transducers. In this talk a review of the properties of transducers-porphyrins couples for gas sensing will be discussed. In particular quartz microbalance, field effect transistors, and optical transducers will be illustrated. A particular emphasis will be given to the development of artificial olfaction systems for which porphyrins-based sensors demonstrated a brilliant attitude as shown for instance by several results in food quality and medical diagnosis.

PL2

ELECTROCHEMICAL MAGNETO-BIOSENSORS FOR THE ULTRASENSITIVE DETECTION OF HORMONES AND BACTERIA

José M. Pingarrón
Department of Analytical Chemistry. Faculty of Chemistry. Complutense University of Madrid. 28040-Madrid, Spain
pingarro@quim.ucm.es

The combination of screen-printed electrodes with functionalized magnetic beads constitutes a powerful and efficient strategy for the development of disposable magneto-biosensors for the rapid and ultrasensitive detection of many analytes of biochemical and clinical significance. Magnetic micro- and nanoparticles have a large active surface area which makes possible the immobilization of a high concentration of biomolecules onto the solid phase of the electrochemical transducer as well as a decrease of matrix effects.

In this talk, some electrochemical magneto-biosensors for the detection of low molecular weight hormones will be presented. Electrochemical immunosensors for the determination of cortisol (1), testosterone (2) and prolactin (3) will be considered.

On the other hand, disposable amperometric magnetoimmunosensors for the specific detection of *Streptococcus pneumoniae* will be also shown (4).

Finally, the preparation of disposable DNA magnetosensors will be discussed. In these designs we integrated the use of electrochemical DNA biosensors with magnetic beads, PCR amplification and the use of disposable screen-printed electrodes. This type of design allowed the development of amperometric magnetogenosensors for the specific detection of a gene related to the *Enterobacteriaceae* bacterial family, based on the coupling of streptavidine-peroxidase to biotinylated *lacZ* gene target sequences [5]. As low as 2.5 aM asymmetric PCR product could be detected with the developed methodology. A further approach involved the use of direct asymmetric PCR amplified products for *E. coli* detection at a concentration level of 1 cfu/100 mL with no need for culture preconcentration steps [6]. Similar strategies were employed to develop DNA sensors for the detection of a characteristic 235-bp region of the gene coding for autolysin (*lytA*), a specific *pneumococcus* virulent factor [7]

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KEYNOTE LECTURES

KN1

PROTEIN-LIGAND INTERACTION STUDIES BY FLUORIMETRIC AND X-RAY SCATTERING APPROACHES

Enrico Dainese[1] and Mauro Maccarrone[1]
[1] *Dipartimento di Scienze Biomediche Comparate, Università di Teramo, Piazza A.Moro, 45, 64100 Teramo, edainese@unite.it*

Nowadays, with the availability of genomes of several organisms, including humans, and the need of identifying, correlating, and understanding, the function of an increasing number of proteins, as well as their interaction with other ligands (small ligands, membranes, other proteins, DNA, RNA, etc.), the interest of ongoing research is focused on the so-called "modular" biology, where the specific biological process of interest is modeled as a complex system of functionally interacting macromolecules within the cell (Maccarrone et al., 2010). Furthermore, a more fundamental understanding of the structure-function relationships of membrane proteins would make invaluable contributions to structural biology, pharmacology and medicine. This research field is extremely important for medical applications because membrane proteins comprise the major target group of modern structural genomics, with approximately 70% of human targets for therapeutic intervention belonging to this class. Thus, the structural and functional study of the protein-ligand interactions is a fundamental step in the knowledge of the molecular mechanisms leading to a biological role of a specific protein system. Here a brief description of the application of fluorescence resonance energy transfer (FRET) and small angle X-ray scattering (SAXS) approaches in this field of research will be presented.

SAXS is one of the most useful methods to analyze in solution the overall structure of macromolecules like DNA, RNA, proteins and lipids. With this technique we can analyze also the ligand-induced conformational changes of proteins, the formation of complexes with specific partner molecules or even the association of a very high number of subunits leading to large assemblies (Dainese et al., 2005). The advantage of SAXS with respect to other techniques in the structural studies of proteins resides in the possibility of performing the measurements in any desired solvent and in the ability to follow changes of the protein structure which may occur as a response to a variety of stimuli: pH or temperature changes, interaction with small ligands, influence of substrate analogues, chemical or genetic modifications, interactions between proteins and/or lipids. This approach is presently undergoing a spectacular expansion associated with the development of powerful data analysis software, with the improvement of the quality of data recorded with synchrotron radiation and with the increasing availability of high resolution tridimensional structures (pdb data base). In this frame, FRET studies are very useful in understanding not only the structural and functional changes induced by membranes on the protein, but also the modulation of the membrane properties due to the interaction with the protein molecule (Dainese et al, 2010).

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KN2

ELECTROCHEMICAL SENSORS AND ELECTROANALYSIS AT THE NANOSCALE

P. Ugo, S. Daniele, L.M. Moretto, M.A. Baldo, D. Battistel, M. Silvestrini
Dipartimento di Scienze Molecolari e Nanosistemi
Università Ca' Foscari di Venezia, Santa Marta 2137, 30123 Venezia
e-mail:ugo@unive.it

This communication presents the research activity developed in recent years at the University Ca' Foscari of Venice, in the field of electrochemical nanosensors and electroanalysis, focusing on the capability to obtain submicrometer sized tools and to apply them for chemical sensing and analytical purposes.
The research activity aimed to prepare electrochemical nanosensors is based on the development of ensembles and arrays of nanoelectrodes.
Ensembles (i.e. random arrays) of nanoelectrodes (NEEs), typically of few tens of nanometer diameter, are prepared by template deposition of metal nanostructures in nanoporous membranes, both commercially available (e.g. track-etched polycarbonate) [1] or custom made, e.g. by suitable magnetron sputtering techniques [2].
Ordered arrays of nanoelectrodes (NEAs) are obtained by top-down techniques such as photolithography in combination with vapor deposition [3] or by electron-beam lithography [4].
NEEs and NEAs are used as high S/N transduction elements in biosensors based on the functionalization of the ensemble/array with antigens [5], DNA or enzymes [6].
Some examples of sensitive analytical applications of NEE/NEA-based biosensors will be presented.
As far as electroanalysis of nanomaterials and/or with submicrometer spatial resolution, recent advances in the field of electrochemical scanning microscopy (SECM) [7] and on the use of macroporous electrodes will be presented.

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ORAL COMUNICATIONS

O1

DETERMINAZIONE MEDIANTE NASO ELETTRONICO DI CAFFE' PROVENIENTI DA AREE GEOGRAFICHE DIVERSE

Sberveglieri V.[1], Concina I.[2], Falasconi M.[2], Gobbi E.[2], Pulvirenti A.[1], Fava P.[1]

[1] *Modena and Reggio Emilia University, Dept. of Agricultural and Food Sciences, Via Amendola, 2 - 42100 Reggio Emilia, Italy.*
[2] *SENSOR, University of Brescia & CNR-IDASC, via Valotti, 9 – 25122 Brescia, Italy*
E-mail: veronica.sberveglieri@unimore.it

In letteratura è frequente trovare lavori atti a distinguere le diverse specie botaniche come ad esempio *Caffea canephora* da *Coffea arabica*, mentre risulta difficile una differenziazione del caffè sulla base della sua provenienza geografica. All'interno quindi della stessa specie botanica, si rivela tutt'oggi una questione difficile se valutata esclusivamente con tecniche chimiche di tipo classico[1].
Il presente studio è diretto a caratterizzare e distinguere il caffè verde e il caffè tostato in base alla sua origine geografica.
Per i produttori risulta arduo ma indispensabile per mantenere elevati standard qualitativi, riuscire a tracciare l'origine geografica delle materie prime alimentari per poter essere in grado di tutelare il consumatore[2].
Le imprese di produzione richiedono strumenti di analisi economici e semplici in grado di fornire una risposta si/no in tempi brevi e possibilmente in modo non distruttivo.
La composizione totale dei composti volatili è determinata e fortemente dipendente da diversi fattori quali le condizioni climatiche in cui vengono coltivate le specie botaniche così come le diverse tecniche per la raccolta e la lavorazione delle materie prima. Il naso elettronico (EN) è stato applicato in diversi campi del controllo alimentare, ed è per questo che abbiamo voluto impiegare questo strumento nella stima della provenienza geografica del caffè. Il naso elettronico (EN EOS^{835}) da noi impiegato per questo studio è costituito da sei sensori di gas a base di film sottili di ossidi metallici semiconduttori ed è equipaggiato con un auto campionatore da 40 posizioni per lo spazio di testa statico. Le analisi effettuate sugli stessi campioni con GC-MS con tecnica SPME sono in buon accordo con i risultati ottenuti dal naso elettronico infatti i componenti volatili, sia per il caffè verde che tostato, evidenziano differenze sia quantitative (diverse percentuali) che qualitative (presenza di composti differenti). I risultati preliminari suggeriscono che il Naso Elettronico non è solo capace di distinguere tra caffè coltivati in paesi diversi ma anche caffè prodotti in regioni diverse della stessa nazione. Nonostante i cambiamenti chimici derivanti dal processo di tostatura questo tipo di tecnica è in grado di discriminare i campioni anche dopo il trattamento industriale.

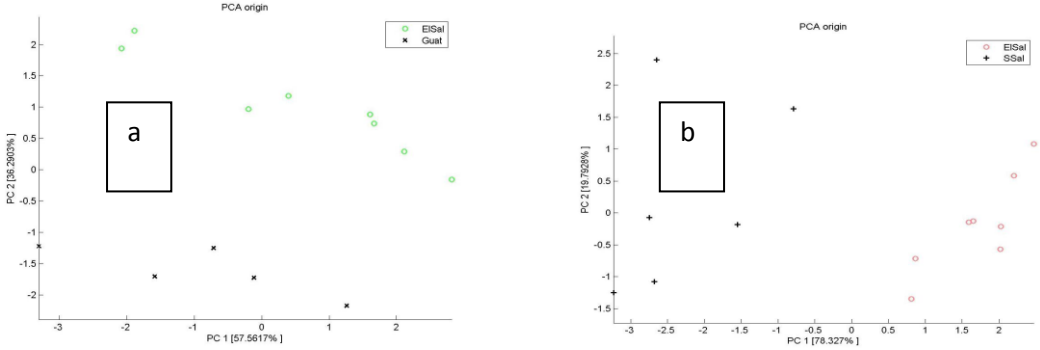

Figure 1 e 2. Risultati ottenuti con PCA (a) nei diversi paesi (cerchi verdi: El Salvador; nero x: Guatemala) e (b) i campioni coltivati in regione diversa di El Salvador (croci nere: distretto della capitale San Salvador; cerchi rosa: El Salvador, nella campagna circostante).

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O2

PEPTIDE MODIFIED GOLD NANOPARTICLES FOR GAS SENSING OF FOOD AROMAS

G.C. FUSELLA*, DEL CARLO M., PITTIA P., D. COMPAGNONE,

Dipartimento di Scienze degli Alimenti, Universita´ degli studi di Teramo, Via Carlo LERICI, 1, 64023 64023 Mosciano Sant'Angelo (TE), Italy

R. PAOLESSE, L. TORTORA

Dipartimento di Scienze e Tecnologie Chimiche ,Universita´degli studi di Roma Tor Vergata, via della Ricerca Scientifica 1, 00133 Roma, Italy

C. DI NATALE

Dipartimento di Ingegneria Elettronica ,Universita´degli studi di Roma Tor Vergata, via del Politecnico, 00133 Roma, Italy

gcfusella@unite.it

It well known that food aromas are among the most important features for customers satisfaction. The addition of volatile compounds during food manufacture or their release of volatiles during the technological steps needs to be monitored to assess the quality of the final product. The resulting final aroma is the result of the entire process [1]. The complexity of the aromas patterns released by food it is also dependent on the composition of the matrix and can be monitored using "electronic noses" [2].

In this study we attempted to add new functionalities to piezoelectric sensors by modification with gold nanoparticles (GNPs) bearing short peptide moieties. GNPs have been synthesized using the $NaBH_4$ method that yelds GNPs in alkaline solution. GNP are unstable due to their high surface energy and need to be stabilized against aggregation by suitable surface modifications. Some functional groups such as cyano (-CN), mercapto (-SH) and amino ($-NH_2$) are known to have an high affinity for gold [3]. The addition in homogeneous solution of compounds as cystein (CYS), glutathione (GSH), γ-glutammylcystein (γ-GLU-CYS) and cysteinylglycine (CYS-GLY) resulted in the formations of modified GNPs. Closely related aminocids were selected to assess the relationships between structure and sensor behavior. The synthesized GNPs have been characterized using TEM, VIS spectroscopy and electrochemistry.

Modified piezoelectric sensors have been then assembled by casting GNPs on 20 MHz quartz crystal microbalances. The response of the sensors array to solvents of different polarity has been characterized by head-space analysis and nitrogen as carrier gas. Model water solutions of aromas used in food addictives as isopentyl acetate, etyl acetate, cis-3-hexen-1-ol and terpinen-4-ol were also tested.

Principal component analysis of data, compared with data obtained using a porphyrin based electronic nose demonstrated that this approach can be useful for the development of a new set of piezoelectric sensors for e-noses.

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O3

IDENTIFICATION OF VIABLE PATHOGENIC BACTERIA BY AN OLFACTORY MOS-BASED SENSOR ARRAY COUPLED WITH FIELD-FLOW FRACTIONATION

Carolina Colliva[1], Barbara Roda[2], Pierluigi Reschiglian[2], Mara Mirasoli[1], Aldo Roda[1]
[1]*Department of Pharmaceutical Sciences, Via Belmeloro, 6, I-40126 Bologna, Italy, carolina.colliva2@unibo.it*
[2]*Department of Chemistry "G. Ciamician", Via Selmi, 2, I-40126 Bologna, Italy*

Every year, almost 80 million cases of food borne illness occur in United States and 1.5 millions in Italy, among these 30% are caused by bacteria and their connected toxic products [1].
Conventional microbiological methods for the identification of food borne pathogenic bacteria including *E. coli* O157:H7,*Yersinia enterocolitica, Salmonella and Listeria* are labor-intensive and time-consuming. Recently, immuno- or nucleic acid-based bioassay allowed significant reduction in the analysis time [2,3]. Nevertheless, their response does not provide information about viability and even dead bacteria are recognized. An approach based on bacteria metabolomics, which greatly differ among species, could be more reliable. Matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS) methods have been described, but due to high cost of instrumentation are not suitable for screening purposes [4]. The metabolomic approach can also be pursued employing electronic olfactory system (EOS), which can detect and classify volatile components, thus enabling rapid bacteria detection [5]. However, EOS are not selective enough to identify bacteria when present in a complex mixture and a preliminary separation is required. Field-flow fractionation (FFF) techniques are separative techniques suitable for the non invasive fractionation of analytes based on their morphological features and therefore useful as pre-analytical tools to add selectivity to EOS approach. This work presents the use of an FFF system coupled with an EOS (EOS 835, SACMI, Imola, Italy) equipped with six metal oxide semiconductor sensors (MOS) for the analysis of volatile metabolites produced by pathogenic bacteria. The sensor technology yields a distinct response signature for each vapour regardless of its complexity, resulting in a "smell fingerprint" which can be used for sample identification by chemometric data analysis. To set up the method, *E. coli* O157:H7 and *Yersinia enterocolitica* were used as model samples. Upon training the EOS with suspensions of each bacteria species (2,4 X 10^9CFU/mL), cells mixtures with different bacteria proportions (1:4; 1:1; 4:1) were injected in an FFF system (final concentration 4,8 X 10^9CFU/mL). Separation is achieved through an empty capillary channel and according to their physical features, cells are swept down the channel at different velocities, i.e. eluted at different times. Fractions corresponding to the retention time typical for the two bacteria species were collected, grown in 1 mL Luria Bertani (LB) broth for 2h at 37°C, and then analyzed with the olfactory system. Each sample was maintained at 37°C and the head space analyzed 5 times (overall analysis time: 2 hours). The data recorded for each sample were averaged and subjected to chemometric analysis using PARVUS software [6]. Feature extraction on the MOS required: 1) a fractional difference calculation (to eliminate baseline drift); 2) selecting the maximum absolute value of the resulting curve and 3) sensor normalization. The selection of 10 variables allowed clearly discriminating by PCA the *Y. enterocolitica* and *E. coli* samples when present in different relative proportion, and the LDA analysis allowed obtaining a correct classification and prediction ability of respectively 90 and 91 %. The analysis of collected fractions from the different mixtures confirmed that after fractionation, the olfactory system was able to distinguish and identify the different fractions. The inter-assay variability is low but a fully highly standardized procedure is required and this is achieved also with the use off the FFF system.
This method could be applied for food safety applications, as well as to biological samples for diagnostic purposes and an on-line FFF coupling with EOS is in progress.

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O4

PORPHYRIN ELECTROPOLYMERS FOR HYPHENATED ELECTROCHEMICAL AND OPTICAL SENSORS

Roberto Paolesse[1], Larisa Lvova[1], Marco Mastroianni[1], Corrado Di Natale[2], Arnaldo D'Amico[2]
[1] *Department of Chemical Science and Technologies, University "Tor Vergata", 00133 Rome, Italy,*
roberto.paolesse@uniroma2.it
[2] *Department of Electronic Engineering, University "Tor Vergata", 00133 Rome, Italy*

The particular properties of porphyrins have recently permitted the development of a variety of chemical sensors based on different transduction principles [1] and among them optodes [2], potentiometric Ion Selective Electrodes [3], chemoresistive [4] and mass sensitive gas sensors [5]. Moreover, the application of electropolymerized thin films of functionalized porphyrins deposited onto conductive and transparent substrate has given the intriguing possibility to develop sensors where two different transduction mechanisms are hyphenated in the same sensing film. This approach can allow a significant increase in the chemical information that can be obtained from the device and it can boost the performances in terms of selectivity and sensitivity.

The pyrrole-functionalized porphyrins are reported in Figure 1. The formation of polymeric film occurred via electrochemical oxidation of the pyrrole substituent groups of the porphyrin monomer. The morphology of the porphyrin electropolymers have been studied in order to clarify how the polymerization mechanism depends on the initial monomer structure, and to evolve the factors influencing the electropolymerisation breakage, as far as the electropolymer sensing properties. Electrochemical methods, UV-visible spectroscopy, SEM and AFM techniques (Figure 2) have been applied for the porphyrin electropolymers characterisation. The single porphyrin electropolymers were successfully applied as hyphenated opto-electrochemical sensors in analytical Computer Screen Photo-assisted Technique (CSPT)-potentiometric platform [6].

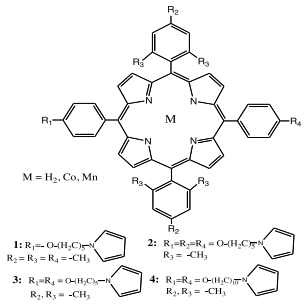

Figure 1. Structures of studied porphyrins.

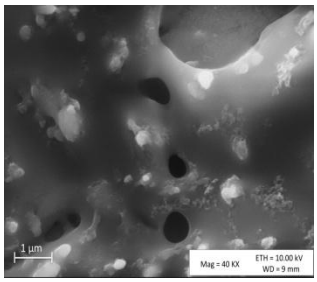

Figure 2. SEM image of porphyrin **3** electro-polymer deposited onto ITO substrate from 1mM CH_2Cl_2 solution of **3** via chronoamperometry (60 sec vs SCE at +0.7 V).

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O5

INNOVATIVE ELECTRONIC OFET BIOSENSORS

Maria Magliulo[1], Maria Daniela Angione[1], Serafina Cotrone[1], Luigia Sabbatini[1], Antonella Mallardi[2], Gerardo Palazzo[1] and Luisa Torsi[1].

[1]*Department of Chemistry ,University of Bari, Via Orabona, 4, I-70126 Bari, Italy.*

[2]*Istituto per i Processi Chimico-Fisici (IPCF), CNR – Via Orabona, 4, I-70126 Bari, Italy*

torsi@chimica.uniba.it

Integration of membranes and proteins into electronic devices involves cross-disciplinary efforts aiming at the full exploitation of a biomolecule specific functional property for advanced bio-electronic applications. Membrane proteins such as ion pumps or receptors, but also hydrophilic ones such as antibodies or enzymes, can be exploited as highly performing recognition elements in electronic sensing devices. Organic thin–film transistors (OTFTs) can allow for simple and low-cost fabrication techniques, miniaturization, multi-parametric responses, signal and response amplification [1] as well as label-free detection are the main features of such devices.

Innovative OTFT biosensors realized through the full integration in the electronic device of the biological recognition elements will be presented. The coupling of the OTFT device and the biological recognition system is actuated by assembling supra-molecular structures in which biomolecules such as membranes and proteins are an integral part of the OTFT active material. Specific reactions (i.e receptor/analyte binding) are then used for analyte detection. To perform the bio-sensing measurements, the solution containing the analyte is deposited on the organic semiconductor, even through a proper microfluidic system. Preliminary results show that such bio-electronic devices can be very selective reaching detection limit (LOD) in the low ppt. In addition, such sensors allow for label-free detection and operate at low reagent and power consumption and can be readily miniaturized and automatized in portable and disposable devices [2].

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O6

DNA BASED MOLECULAR SWITCHES FOR THE DETECTION OF ANTIBODIES AND TRANSCRIPTION FACTORS

Francesco Ricci[1], Alexis Vallee-Belisle[2], Andrew J. Bonham[2] Kevin W. Plaxco[2], Giuseppe Palleschi[1]
[1] *Dipartimento di Scienze e Tecnologie Chimiche, Università di Roma, Tor Vergata, Via della Ricerca Scientifica, 00133, Rome, Italy. Email: francesco.ricci@uniroma2.it*
[2] *Department of Chemistry and Biochemistry, University of California, Santa Barbara, Santa Barbara, CA 93106 USA*

Here we report the development of two versatile DNA-based switches which enable single-step quantitative detection of antibodies and transcription factors through direct optical or electrochemical outputs triggered by binding-induced structural changes.
In the case of antibody detection, we designed a DNA-based nanoswitch that is triggered by binding to two distinct sites on a single target macromolecule. By coupling this bidentate nanoswitch to optical and electrochemical outputs, we achieve the rapid (seconds/minutes), quantitative detection of sub-nanomolar concentrations of Ab raised against many antigens (*e.g.,* small molecules, peptides etc) even in highly complex samples, such as whole blood. Antibody beacons could be easily implemented in inexpensive, easy to use, electronic hand-held devices, suggesting that they may be particularly well suited for point-of-care applications.
We also developed a novel class of DNA-based structure-switching probes, which we have termed "transcription factor beacons", that enable, for the first time, the quantitative detection of DNA binding activity directly in complex biological samples. TF beacons are reagentless, and binding-activated, which drastically simplifies detection of DNA binding activities by removing washing, transfer, or electrophoresis steps. Furthermore, the high selectivity of their signaling mechanism enables DNA binding activity detection directly in complex biological samples. TF beacons can be adapted for the detection of any DNA binding activities without the need to generate specific antibodies. Given these attributes, we believe that TF beacons will enable important advances in applications ranging from drug screening, cancer diagnostics, and developmental biology, where interest in the quantitative regulation of TFs is growing.

O7

AFFIBODY MOLECULES: A NOVEL CLASS OF AFFINITY LIGANDS FOR TUMOUR MARKER IMMUNOSENSORS

Hoda Ilkhani[1], Marco Mascini[2], Giovanna Marrazza[2]
[1]*Center of Excellence in Electrochemistry, University of Teheran, Enghelab street 1, Teheran, Iran*
[2]*Dipartimento di Chimica "Ugo Schiff",Università degli Studi di Firenze,*
via della Lastruccia 3, 50019 Sesto Fiorentino, Firenze, Italy
giovanna.marrazza@unifi.it

The development of immunosensors for the detection and monitoring of cancer markers is currently a major area of research and, as more markers are discovered and their role in disease becomes better understood, this will continue to grow. The success of these assays is largely due to the properties of the antibodies used to capture and detect the analyte, due to their high sensitivity and specificity. However, they are not without disadvantages and this has led to investigation into alternatives. Affibody molecules are one such option currently being researched. They are a small robust three helical peptide, made up of only 58 amino acids that contain no disulphide bonds and can therefore be produced in simpler organisms, such as prokaryotes, rather than the animal systems required in antibody synthesis.
The goal of this work was to design a sandwich immunoassay based on affibodies as bioreceptors for the detection of a cancer marker, human epidermal growth factor receptor 2 (HER2), with electrochemical transduction.
HER2 (also known as neu, ErbB-2, ERBB2) stands for "Human Epidermal growth factor Receptor 2" and is a protein giving higher aggressiveness in breast cancers.
The immunosensor is based on a sandwich format in which a primary monoclonal antibody anti-HER2 is coupled to protein A modified magnetic beads. The modified beads are then used to capture the protein from the sample solution and the sandwich assay is performed by adding the affibody labelled with biotin. The enzyme alkaline phosphatase (AP) conjugated with streptavidin is then incubated with the complex on the beads. After, the modified magnetic beads are captured by a magnet on the surface of a graphite working electrode and the electrochemical detection is thus achieved through the addition of the AP substrate (□-naphthyl-phosphate) and □-naphthol produced during the enzymatic reaction is detected using differential pulse voltammetry (DPV).
The optimal conditions for affibody molecules have been explored to ensure that the best performance of the immunosensor. The performance of the bioassay in terms of sensitivity, reproducibility and selectivity has been studied in buffer and serum samples.

O8

MULTIPLE ELECTROCHEMICAL DETECTION OF DNA HYBRIDIZATION BY 2-ELECTRODES DEVICE

Simone Bonetti[1], Daniele Gazzola[1,2,3], Manuele Onofri[1], Giampaolo Zuccheri[1,3], Bruno Riccò[2], Bruno Samori[1,3]

[1] *Department of Biochemistry G. Moruzzi, University of Bologna*

[2] *Department of Electronics, Computer Sciences and Systems DEIS, University of Bologna*

[3] *Industrial Research on Health Science and Technology (ICIR-HST)*

Electrochemical biosensors are amongst the most promising devices for the detection of biomolecules. The call for cheap, automated, miniaturized and parallel biosensors leads to possible adaptations of more classical electrochemical techniques. In this communication, we report on the implementation of a two-electrode electrochemical biosensor (Fig. 1) for the detection of soluble nucleic acids via hybridization on the electrode surface. The use of a soluble redox label that binds double-stranded DNA specifically (HOECHST 33258) enables the detection of DNA oligonucleotides down to at least a 1 nM concentration (Fig. 2).

As we have demonstrated, binding of Hoechst 33258 is irreversible, thus only one measurement per electrode is possible. In a three-electrode set-up, this would imply that only one experimental data point can be gathered for each electrode set. In our two-electrode set-up, instead, one of the two same electrodes has been set to a negative potential while the Hoechst 33258 was oxidized at the other electrode. As a consequence of this, all the dye bound to the DNA at that electrode is still in a reduced state and can thus be oxidized in a second measurement, when the same potential sweep is applied to the second electrode with respect to the first. In this communication, we define the first measurement performed on a biochip *direct measurement*, and the second one *inverse measurement*. In Figure 3, we show the traces of the direct and inverse measurements of three devices performed on buffer containing 10 nM target DNA. The current of the oxidation peaks resulting from the inverse measurements (grey lines) is comparable to the current peaks from the direct measurements (black lines), and also the repeatability is similar. The practical advantage of the quantitative match between direct and inverse measurements lies in the possibility of performing a 'control measurement' that can cross-check and confirm the results of the direct one, thereby compensating for experimental errors and resulting in an improvement of the reliability of the biosensor. Alternatively, if the two electrodes can be derivatized with SAMs of different probe oligonucleotides, the two measurements could quantify the presence of two different nucleic acids analytes in the same flow, leading to the best possible utilization of the electrodes: only one electrode per target would be necessary.

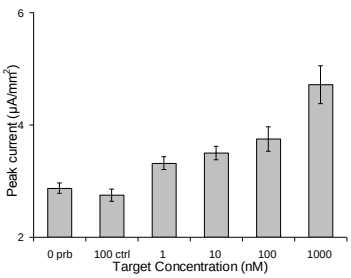

Figure 2. Calibration curve of the biosensor from 0 to 1 uM. The "100 ctrl" column refers to 100nM of non complementary sequence

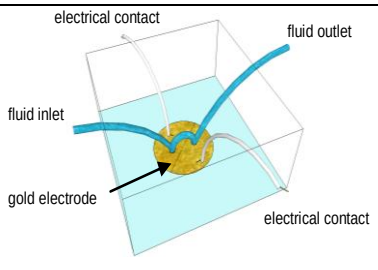

Figure 1. Two-electrode electrochemical biosensor with the microfluidic chamber.

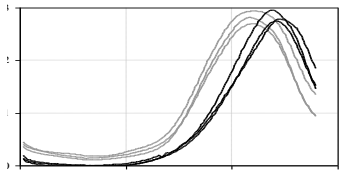

Figure 3. Direct (black line) and inverse (grey line) measurements performed on buffer containing 10 nM target DNA.

O9

APPLICATION OF THE THICKNESS SHEAR MODE (TSM) METHOD FOR DNA APTAMERS-BASED BIOSENSOR DEVELOPMENT AND OCHRATOXIN A DETECTION

T. Hianik[1], G. Castillo[1], I. Lamberti[2], L.Mosiello[2]
[1]*Department of Nuclear Physics and Biophysics, Comenius University, Bratislava, Slovakia.*
[2]*ENEA, Italian National Agency for New Technologies,Energy and the Environment Via Anguillarese 301, 00060 Rome, Italy, lucia.mosiello@enea.it*

We developed aptamers based biosensor for label-free detection of ochratoxin A (OTA) by thickness shear mode acoustic method (TSM), using experimental set-up as described in ref. 1. This method is sensitive to mass adsorbed at the sensor surface, measured by changes of resonant frequency, f_s, and to the viscoelastic contribution, measured by motional resistance, R_m. Addition of OTA to a sensor surface with immobilised biotinylated DNA aptamers resulted in decrease of f_s and increase of R_m. We were able to detect OTA with limit of detection of 30 nM and determined the equilibrium dissociation constant K_D=43.9±30 nM. The OTA interacted with aptamer only at presence of calcium ions. Therefore binding studies were performed at presence of 20 mM Ca^{2+}. No significant changes of f_s and R_m were observed without calcium. We analyzed also changes of acoustic parameters at presence of possible interference N-acetyl-L-phenylalanine (NAP). Addition of NAP in a concentration range 25-740 nM resulted similar frequency changes like that induced by OTA, however significant but much lower changes of motional resistance were observed only at highest NAP concentration analyzed (740 nM).
We showed that TSM acoustic method is perspective tool for label-free detection of low molecular toxicant – OTA using DNA aptamers as specific receptors. The detection of OTA with aptamers requires presence of calcium ions that are most probably responsible for stability of aptamer and for formation of 3D structure of specific binding site for OTA. The analysis of thermodynamic properties of aptamers certainly indicate on increased melting temperature of aptamers, and hence increased stability, with increased concentration of calcium in a range 0-20 mM. The limit of the detection of the biosensor (30 nM) was comparable with QCM based acoustic sensor, but utilizing indirect, competitive assay.
In contrast with other authors [2], we observed certain interference of aptamers sensor with NAP that affects significantly the frequency, but not the motional resistance. It is possible that NAP interact non-specifically with DNA or with neutravidin layer. Therefore further optimization of TSM sensor will be necessary.

Acknowledgements:
This work was supported by Agency for Promotion Research and Development under the contracts No. APVV-0362-07, by Slovak Grant Agency (Project No. 1/0794/10) and by scholarship program of SAIA.

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O10

ENZYME-BASED AND ENZYME-FREE GLUCOSE SENSORS

Fabio La Mantia
Centre for Electrochemical Sciences, Ruhr-Universität Bochum (Germany)
fabio.lamantia@rub.de

Glucose sensing is one of the success stories of biosensing. The health and the life quality of diabetes patients depend on the accurate monitoring of their blood glucose levels by means of glucose biosensors. The main challenges in enzyme-based glucose sensing are the low overall catalytic (volume) density, the need of artificial redox mediators, the limited life time, and a strong dependence of the catalytic activity on the environment.

To overcome partially the above mentioned limitations reagentless biosensors were proposed in which all compounds necessary for the generation of the signal are tightly immobilized at the sensor surface. In order to design a fast electron-transfer pathway between the prosthetic group of the biological recognition element and the electrode, Os-complex modified electrodeposition polymers were synthesized and used as immobilization matrix for PQQ-dependent glucose dehydrogenase. The polymer backbone as well as the redox potential of the polymer-bound Os complex were optimized to enable a high sensitivity [1].

Despite the strategy of designing optimized reagentless biosensors with tunable properties was successfully accomplished and a comparable high amount of active enzyme could be immobilized on the electrode surface, the intrinsic limitations of enzyme-based sensors such as the short operational stability remained.

This is especially important for implantable glucose sensors. Therefore, we have considered the direct oxidation of glucose on gold surfaces. The complex oxidation of glucose at the surface of gold electrodes was studied in detail in different conditions of pH, buffer and halide concentration. An *oxidative peak in the cathodic scan* was observed in the cyclic voltammetry of glucose at gold electrodes, its peak current density being proportional to glucose concentration in a wide potential range. The application of this phenomenon in blood glucose sensing has been prevented by the presence of inhibitors such as chloride. In order to overcome this problem, the mechanism for the formation of the oxidative peak has been investigated [2]. The understanding of the mechanism was the basis to develop a strategy to overcome the inhibition to glucose oxidation induced by the chlorides has been postulated and investigated [3]. It has been demonstrated that it is possible to detect glucose under blood-like conditions (pH and chloride content) by a pulsed amperometric technique.

Acknowledgement
The author thanks dr. Dmitrii Guschin, Minling Shao, Xingxing Chen, Sascha Pöller, and Prof. Dr. Wolfgang Schuhmann for the fruitful discussion and support.

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O11

NEW IMMUNOSENSORS OPERATING IN ORGANIC PHASE (OPIEs) FOR ANALYSIS OF TRIAZINIC PESTICIDES IN OLIVE OIL

Mauro Tomassetti, Elisabetta Martini, Luigi Campanella
Dipartimento di Chimica, Università "La Sapienza", P.le Aldo Moro 5, 00185 Roma, Italy

The chemical analysis of matrixes scarcely soluble or completely insoluble in aqueous solutions has always posed a serious problem for the analytical chemistry, which has only been partially solved by such techniques as gas chromatography or head space analysis. Biosensor analysis has recently made a substantial contribution to solving this problem through the development of OPEEs, i.e. enzymatic electrodes capable of operating in organic solvents. One classical example is that of inhibition OPEEs to analyse different types of pesticides that are relatively insoluble in aqueous solution, in the development of which also our team has recently been involved [1-3]. The drawback consists in the fact that it is often complained that inhibition biosensors are relatively unselective, also versus pesticides belonging to different phytopharmaceutical classes. It is a known fact that immunosensors are the most selective biosensors, and our team has recently fabricated immunosensors for triazinic pesticides determination, although operating only in aqueous solution [4]. However the greater knowledge acquired in recent times of the, often widely differing, effects exerted by different organic solvents on the protein compounds dissolved in them has now encouraged several authors to test the possibility of developing antibody-based or similar methods using different solvents or mixtures of organic solvents, mainly water-alcohol mixtures. With this in mind we undertook a research aimed at developing immunosensors for triazinic pesticides analysis in a hydrophobic matrix such as olive oil operating in (non alcoholic) mixtures of organic solvents, for instance, chloroform - hexane 50% V/V mixture, in other words, in a mixture that had previously proved to be particularly suitable when enzymatic OPEEs were being developed.

In particular, an immunosensor for atrazine was tested in which a hydrogen peroxide electrode was used as transducer and peroxidase as marker. The competition process took place in the chloroform-hexane mixture described above, while the subsequent enzymatic measure was performed in an aqueous buffer solution. A linear response of between about 0.05 and 5 µM was obtained versus atrazine. Subsequently, an attempt was made to use a Clark electrode as transducer and to perform not only the competition but also the final enzymatic measure in organic solvent. It was attempted to carry out the latter measure in different organic solvents such as decane, hexane, chloroform and chloroform-hexane mixture. Up to today the best analytical results were obtained using decane as solvent.

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O12

DIRECT AND MEDIATED ELECTROCHEMICAL APPROACHES FOR THE ANALYSIS OF POLYPHENOLS IN EXTRAVIRGIN OLIVE OIL

Del Carlo M.[1], Fusella G.C.[1], Della Pelle F.[1] ,Cichelli A.[2], Amine A.[3], Compagnone D.[1]
[1] *Dipartimento di Scienze degli Alimenti, Università di Teramo*
[2] *Dipartimento di Scienze, Università D'Annunzio Chieti-Pescara*
[3] *Université Hassan II, Mohammedia, Marocco*

Virgin olive oil is obtained from the fruit of the olive tree (Olea europaea L.) solely by mechanical or other physical means under conditions that do not alter its properties and must not undergo any treatments other than washing, decantation, centrifugation, or filtration. These processes maintain volatile and other minor compounds such as phenols that enhance the characteristic flavor of virgin olive oil. Stability is not a standard parameter used to measure quality. However, it provides information about the hypothetical shelf
life of the oil. In particular, lower stability indicates a poorer quality (e.g., greater acidity, higher peroxide values and extinction coefficients, and lower sensorial score). It has been shown that 78% of the stability, evaluated by Rancimat, is due to the combined effect of two variables, namely, phenolic compounds and the oleic/linoleic (O/L) ratio.
The phenolics of virgin olive oil (VOO) belongs to several classes but the secoiridoids are the very abundant compounds. Among the last the o-diphenols molecules (oleuropein aglycon, decarboxymethyl oleuropein aglycon and hydroxytyrosol) are the most potent antioxidants. Phenols, and especially o-diphenols, are easily oxidized on the surface of polarized carbon electrode. The most readily oxidizable compounds are also the molecules that give more easily electrons. This behavior reflects the different anodic potential able to oxidize single phenolic compounds. Analysis by flow injection analysis-amperometry, cyclic voltammetry and hydrodynamic voltammetry both on phenolic extracts and phenolic molecules collected by semi-preparative liquid chromatography, were carried out. Information about phenols obtained from electrochemical techniques were compared with several chemical data. Also the antioxidant and radical scavenging activity of these has been evaluated.
Moreover the use of an sodium molibdate has been evaluated to selectively measure, via electrochemical approach, o-diphenols in olive oil extracts. The molibdate has been used in solution and immmobilised on carbon nanotubes. Among carbon nanotubes, both single and multiwall carbon nanotubes have been used to immobilize the molibdate. Both –COOH and $–NH_2$ functionalization of SWCNT and MWCNT have been tested.
An appreciable sensor stability was observed for the $–NH_2$ functionalization especially in MWCNT. A significant increase in sensitivity was observed using nanostructured electrodes as compared with graphite screen printed electrodes.

O13

OPTICAL pH NANOSENSING WITH MODIFIED CARBON NANOTUBES

F. Baldini[1], C. Bianchini[2], G. Ghini[1], G. Giambastiani[2], A.Giannetti[1], L. Luconi[2], G.L. Puleo[2], C.Trono[1]
[1]IFAC-CNR, Via Madonna del Piano 10, Sesto Fiorentino, Firenze, 50019, Italy
[2]ICCOM-CNR, Via Madonna del Piano 10, Sesto Fiorentino, Firenze, 50019, Italy
ghini@ifac.cnr.it

Three different ethylen-glycol fluorescein derivates, Fluo1, Fluo2 and Fluo3, were bound covalently to carboxyl functionalized MWCNTs (Figure 1).

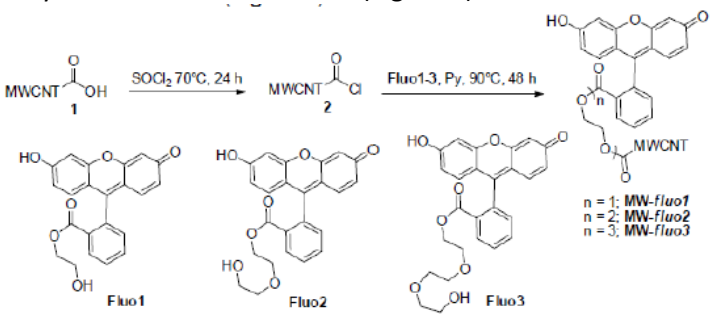

Figure 1. Reaction scheme for the preparation of fluorescently labeled MWCNT.

TGA analysis allowed evaluating roughly the content of fluoresceine ester anchored on MWCNT: 4.9 wt% and a 5.5 wt% have been found for the MW-fluo2 and MW-fluo1 samples, respectively, while only a 1.6 wt% of the dye-ester Fluo3 grafts the MWCNT side-wall. The pH dependence of the modified nanotubes was investigated interrogating, after suitable sonication, an aqueous solution of MWCNT-derivates, in the 4 to 9 pH units range. Dye excitation was obtained by means of a laser diode with emission peak centered at 488 nm. An optical fiber (core diameter = 1 mm), positioned at 90° with respect of the excitation beam direction, is connected with an ANDOR optical spectrograph for the recording of the fluorescence spectra. Figures 2 show the fluorescence spectra of MWCNT2 for different pH values and the related calibration curve, respectively. The modified MWCNTs exhibited linear pH dependence in the range between 5.5 pH and 7.5 pH units with a sensitivity less than 0.1 pH units.

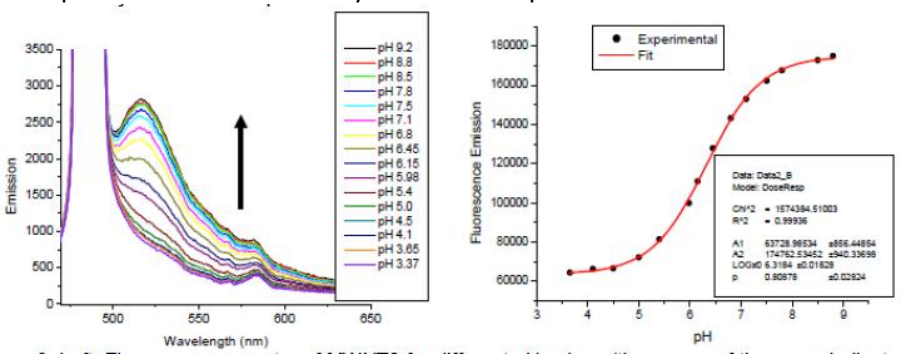

Figure 2. Left: Fluorescence spectra of MWNT2 for different pH values (the versus of the arrow indicates an increase in the pH value. Right: pH calibration curve. The fitting with a sigmoidal curve, of the optical intensity detected between 510 nm and 650 nm (dots), is also shown.

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014

SEPSIS ANALYSIS WITH THE CHANNEL ARRAY INTERROGATION INSTRUMENT

Ambra Giannetti[1], Cosimo Trono[1], Luca Bolzoni[2], Giampiero Porro[2], Francesco Baldini[1]
[1]IFAC-CNR, Via Madonna del Piano 10, Sesto Fiorentino, Firenze, 50019, Italy a.giannetti@ifac.cnr.it
[2]Datamed S.r.L., Via Papa Giovanni XXIII 45, Rodano, Milano, 20090, Italy

In the five-year integrated project CARE-MAN, a point of care (POC) device was designed, developed and implemented. The basis of POC devices is the need of physicians to have a fast and reliable response to formulate the right diagnosis or to decide the correct therapy, avoiding to deliver the samples to the central laboratories and to wait for a period of time, generally several hours long, to achieve the results of the analysis. The quick discrimination of viral and bacterial sepsis in intensive care patients or the fast identification of the origin of infections can be essential for patient's life and consequently this is one of the cases in which POC instrumentation can play a fundamental role. Sepsis can be caused by numerous pathogens and the primary state of infection can be in any major organ system. Therefore a single marker could not provide the high accuracy needed for fast and accurate guidance of treatment of sepsis patients, and a combination of markers should be considered the right approach. Several are the potential biomarkers necessary to define the etiology of severe infections, such as C-reactive protein (CRP), procalcitonin (PCT), tumour necrosis factor α, myeloperoxidase, interleukine-6, interleukine-8, interleukine-10 and neopterin. For this reason a channel array interrogation (CAI) system was developed (Figure 1). The heart of the system is the chip shown in Figure 2. It is constituted by a two-piece polymethylmetacrylate (PMMA) chip, with microchannels through which the analysed sample flows. The cross section of each microchannel is rectangular, 500 µm wide and 50 µm high. The sensing layer, where the immunochemical reaction takes place, is located on the bottom side of the upper piece of the PMMA chip. The exciting radiation, perpendicular to the sensing layer, comes from a laser diode emitting at 635 nm, properly filtered with a band pass filter at 635 nm and focused by means of a cylindrical lens. The emitted fluorescence, which comes from the sensing layer when the specific biologic interaction takes place, is mainly coupled to the PMMA cover of the chip. The particular shape of the chip cover, with a comb-shaped cross-section with air gaps, creates plastic waveguides which runs side by side to the flow channels. The fluorescent signal is laterally collected by a single plastic optical fibre connected to a Hamamatsu optical spectrum analyser. A high-pass (HP) filter, cut on at 650 nm, takes care to cut the light coming from the source and scattered by the PMMA chip.

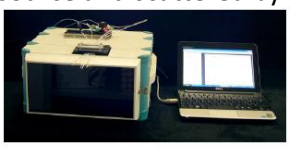

Figure 1. The CAI instrument

Figure 2. The 13-channel chip

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O15

A PORTABLE MICROFLUIDIC DEVICE BASED ON CHEMILUMINESCENCE "CONTACT" IMAGING FOR DETECTING AND GENOTYPING PARVOVIRUS B19

Luisa Stella Dolci[1], Mara Mirasoli[1], Francesca Bonvicini[2], Angela Buragina[1], Francesca Di Furio[2], Massimo Guardigli[1], Aldo Roda[1]

[1] *Department of Pharmaceutical Sciences, Alma Mater Studiorum, University of Bologna, Via Belmeloro 6, 40126 Bologna, Italy*

luisastella.dolci2@unibo.it

[2]*Department of Haematology and Oncological Sciences "L. e A. Seragnoli", Microbiology Section, University of Bologna, Via Massarenti 9, 40138 Bologna, Italy*

The development of miniaturized analytical devices is fundamental for Point-Of-Care (POC) applications, near the site of patient care rather than in the traditional central laboratory. Features of a POC device should include portability, ease of use, simple or no sample pre-treatment, and short assay time associated with a high sensitivity

Parvovirus B19 (B19V), which is mainly transmitted through respiratory route, is responsible for a wide spectrum of pathologies and, most remarkably, it can lead to severe consequences such as fetal death if infection is contracted in pregnancy [1]. An rapid and early diagnosis of B19V infection is thus needed to avoid potential fetal risks.

In this work, a miniaturized analytical device based on chemiluminescence (CL) "contact" imaging CCD detection [2] was developed for the diagnosis of Parvovirus B19 (B19V) infection and identification of the viral genotype through the detection of viral DNA in serum samples. Oligonucleotide probes specific for B19V genotypes were covalently immobilized (1mm^2/spot) in separate positions on a glass support, which was coupled with a polydimethylsiloxane (PDMS) fluidic element, which also contained reservoirs for samples and reagents loads and adsorbent pads. Reagents flow through the channels (0.1x1x35 mm) was driven by capillary force, without use of pumps. The strategies for the covalent immobilization of oligoprobes onto the glass surface and for the reduction of non-specific binding were carefully optimized to increase reproducibility and signal-to-noise ratio. Biotin-labelled target molecules were captured by immobilized oligonucleotide probes, then quantified using a streptavidin-horseradish peroxidase (HRP) conjugate and "contact" CL imaging using a cooled CCD upon addition of a suitable HRP CL substrate.

The performance of the analytical device was evaluated by employing synthetic biotinylated oligonucleotide targets correspondent to the three B19V genotypes (Figure 1). Limits of detection (50 fmol/mL for each B19V genotype) comparable with those obtained using standard laboratory techniques were achieved, with an overall assay time of 10 min and negligible cross reaction between the different genotypes. This system is currently under evaluation for the detection of B19V DNA in clinical serum samples after PCR amplification. Miniaturized PCR systems and isothermal PCR are also being investigated to include the PCR step in the portable device.

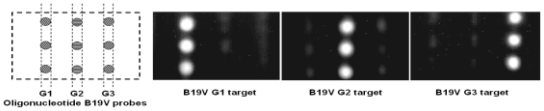

Figure 1. CL images obtained with the miniaturized analytical device for the three B19V genotypes (left: immobilization layout of the specific oligonucleotide probes).

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O16

MICRO ELECTRODES ARRAY (MEA) TECHNOLOGY: A MULTI-BIOMEDIATOR AND MULTI-DETECTION BIOSENSOR FOR AGRIFOOD AND ENVIRONMENTAL MANAGEMENT

[1,2]Viviana Scognamiglio, [3]Christos Boutopoulos, [1]Teresa Lavecchia, [1]Giuseppe Rodio, [2]Gianni Pezzotti, [2]Ittalo Pezzotti, [3]Ioanna Zergioti, [1]Maria Teresa Giardi

[1] *Institute of Crystallography, CNR, 00016 Monterotondo, Rome, Italy*

[2] *Biosensor Srl, Via degli Olmetti 44, Formello, Rome, Italy*

[3] *National Technical University of Athens, Physics Department, 15780 Zografou, Athens, Greece*

According to the report "Biosensors in Medical Diagnostics: A Global Strategic Business Report" published by Global Industry Analysts Inc., the global market has grown from $6.1 billion in 2004 to $8.2 billion in 2009, and it will continue at an average annual growth rate of 6.3% in 2010/11 (1). Although the research activity on biosensor technology is considerable, their applications in agrifood and environmental fields and their commercialisation remain still limited. One reason can be ascribed to the lack of ability to discriminate among various compounds in the same real sample, since the simultaneous presence of different compounds represents a real challenge in the detection of a specific analyte (2). The development of a biosensor system based on an array of different biological recognition elements, able to monitor a wide range of compounds, and the employment of several transduction systems integrated together to create a biosensing platform, could be an useful strategy to overcome this effort. The micro electrodes array (MEA) technology represents a useful tool to project innovative biosensing systems which employ enzymes, receptors and microorganisms as biomediators for water and food constituents and contaminants, associated to amperometric, conductometric and optical transductors integrated in a biodevice with high performance in the analysis of agrifood and environmental samples. Our laboratory, in collaboration with an industrial partner (Biosensor s.r.l., www.biosensor.it), projected and realised a multi-biomediator multi-transductor biosensing device based on MEA technology. The device is composed of an array of 64 gold electrodes on which biomediators are immobilised by the LIFT (Laser Induced Forward Transfer) technique, with 10-30 µm dimension spots and a spatial resolution of 1 µm. Each biomediator is associated with a different transductor to elaborate the signals from the catalytic or binding processes. The electrodes are connected to an electrochemical module for the amperometric and conductometric parameters set-up, and of an optical module, composed by LEDs and photodiodes, for the excitation and fluorescence emission measurement. Receptor proteins and oxidase/dehydrogenase enzymes are employed for the detection of the main food constituents, such as sugars, amino acids, polyphenols, lipids, while photosynthetic microorganisms and cholinesterase enzymes to monitor pesticides in water and food samples, with limits of detection in nanomolar and micromolar ranges.

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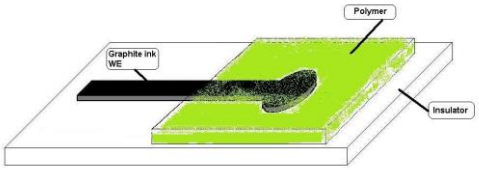
O17

ALL-SOLID STATE ION SELECTIVE ELECTRODE FOR DOPAMINE BASED ON MOLECULAR IMPRINTED POLYMER

Maria Pesavento, Girolamo D'Agostino, Daniele Merli
Dipartimento di Chimica, Università di Pavia, via Taramelli 12 27100 Pavia, maria.pesavento@unipv.it

Molecular imprinted polymers are synthetic copolymers containing recognition sites that mimic the molecular recognition properties of natural receptors, with advantages in terms of robustness, reproducibility, low cost, and easy integration on conducting surfaces. For these reasons they are widely employed in electrochemical sensors (1) with amperometric and voltammetric transduction. Only in a few cases potentiometry was used as discussed in a recent review (2).

In principle the integration of imprinted polymers with potentiometric transduction has interesting potentialities in the fabrication of sensing devices suitable for on–site monitoring and field studies. Besides, a unique feature of potentiometric techniques with respect to the amperometric ones is that the species does not have to diffuse through the membrane, so that the equilibration time is reduced. For example our group has proposed for the first time a potentiometric sensor for atrazine based on the selective rebinding of atrazinium ion to a selective MIP in the form of a rigid membrane implemented in a conventional potentiometric device with inner filling solution in contact with Ag/AgCl electrode (3). A LOD of around 10 μM was obtained with very good selectivity and fast response, and a non nernstian sensitivity of around 27 mV/dec. One of the drawbacks of classical ISEs, as that described above for atrazine, is that they are hardly miniaturizable, because of the inner reference electrode Ag/AgCl requiring a chloride filling solution. Moreover the detection limit is usually determined by the analyte concentration in the inner solution. For this reason all-solid-state ISE have been proposed (4), a schematic view of which is reported in figure:



In the present work an all solid state ISE for the organic cation dopame was prepared, similar to that previously proposed for atrazine (3). An homogeneous MIP membrane was obtained by in situ polymerization procedure, as previously described (3). In the present case the polymer was synthesized directly on a carbon ink screen printed electrode, by copolymerization of methacrylic acid (as active monomer) and ethylene glycol dimethacrylate (as cross linker) in the presence of dopamine hydrochloride as template.

It must be recognized that the selective membranes here employed are similar to those used for usual ISEs in that they too contain a ionophore, which is the selective site obtained by the imprinting technology. The potential was measured against a double junction Ag/AgCl reference electrode, obtaining a non-nernstian sensitivity of 30 mV/dec at DA concentration higher than about 10 μM. The response time was as high as several hours, with a large drift.

A substantial improvement of the equilibration time was obtained by modifying the carbon ink surface with a thick layer of multiwalled carbon nanotubes (MW-CNT) (Cheap Tubes inc) before application of MIP membrane, to assure an effective ion to electron transduction (4). This approach was successfully used for a conventional K-ISE based on valinomycin as ionophore (5).

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O18

BIOCATALYTIC NON WOVEN NANOFIBROUS MEMBRANES (NW- NFMs)

Matteo Scampicchio, *Free University of Bolzano-Bozen, Faculty of Science and Technology*
Saverio Mannino, S. Benedetti *University of Milan, Department of Food Science and Technology*

Non Woven Nanofibrous Membranes prepared by electrospinning represent a new and innovative "nano" support which can be used to coat electrochemical sensors to enhance their selectivity and adsorption capability.
The use of such nano membranes has several advantages respect common thin or thick coatings. First, the membranes have low mass transport resistance. Either the solvent or the molecules can pass freely toward the channels of the membranes and reach the electrode surface with negligible diffusion rate changes. However, the functionalities of the membranes can be modify in a way that they hinder the diffusion of certain class of molecules, e.g. by chemisorptions or physisorption process. This selectivity is very attractive and it can find application to remove interferences from complex samples.
Second, the "nano" structure of the membrane offers a very large surface area available, which can enhance the adsorption loading of inorganic catalysts or enzymes. Higher loading of enzyme means higher signal and lower detection limit.
Third, the nanostructured coating can be used to protect the electrode surface from passivation. Hydrophobic and hydrophilic nature of the membrane can be used to keep away undesired compounds. Such behavior can be easily modified by functionalizing the surface functionalities of the fibers or by modifying the solvent properties (e.g. pH, dielectric constant, etc.).
In conclusion, the NW-NFMs Sensors will combine the properties of both EC sensors and NW-NFMs. Since the most evident property of the NW-NFMs is their superior surface available respect thin or thick films, the way to modify and control its surface functionality can allow the design of new selective electrochemical sensors and biosensors with unmatched reactivity.
In this presentation the new knowledge on the existing interaction between the nanostructure environment of the NW- NFMs and the transduction capability of the resulting sensor will be discussed.

O19

ELECTROCHEMICAL SENSORS BASED ON ELECTRODES COATED WITH LAYERED DOUBLE HYDROXIDES

Lorella Guadagnini, Isacco Gualandi, Adriana Mignani, Marco Monti, Erika Scavetta, Domenica Tonelli
Dipartimento di Chimica Fisica ed Inorganica, Università di Bologna, Viale Risorgimento 4, 40136 Bologna,

Layered Double Hydroxides (LDHs) have general formula $[M_a(II)_{1-x}M_b(III)_x(OH)_2]^{x+}(A^{n-}_{x/n})\cdot mH_2O$, where x ranges between 0.2 and 0.5, depending on the nature of the M_a and M_b metals [1]. These materials are attractive due to their anionic exchange behaviour which makes them useful in several fields, such as catalysis, separation technology, and medicine. When $M_a(II)$ is a transition metal, like Ni or Co, undergoing a reversible redox reaction between the oxidised and reduced states, the material displays improved charge transport properties, especially in alkaline solution [2]. For most LDHs applications in the field of electrochemical sensors, it is required to coat a metal or a carbon-based substrate with a stable thin film. Previously, we have described a simple and rapid electrochemical procedure to deposit, with good reproducibility, well adherent thin films of Ni/Al or Co/Al LDHs on conductive surfaces [3].

In this contribution we report two analytical applications of cobalt-based LDH modified Pt electrodes. The former concerns the development of a potentiometric pH sensor, the latter the amperometric detection of salicylic acid (SA). The performances of the coated electrode as pH sensor are summarized in Figure 1. It shows, as a function of time, the recorded potential and the corresponding pH value, simultaneously recorded with a conventional glass electrode, in a dynamic experiment where the pH of a universal buffer solution is increased in the range 5.5-12 by dropwise addition of KOH. The graph highlights the excellent behaviour of the new sensor, as to response time (5 s for all the investigated pH values) and signal stability. The response is linear, with a slope of -76.2 ± 0.6 mV/pH in the pH interval between 2 and 14, and does not suffer from memory effects.

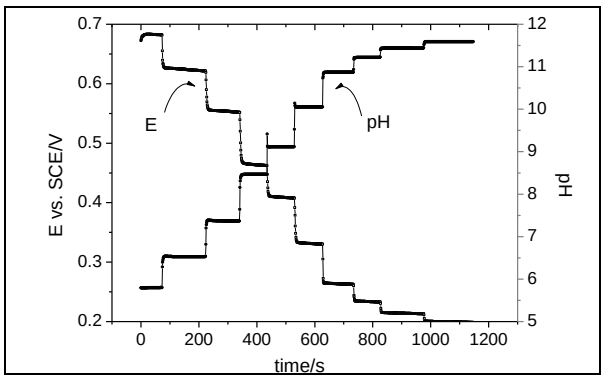

Fig. 1. E vs. time response of the Co/Al-LDH Pt sensor and pH response of a glass electrode

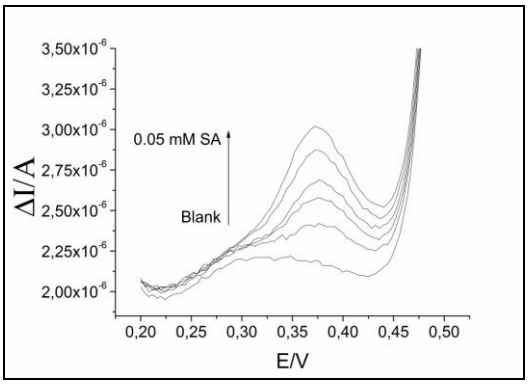

Fig. 2 DPV responses for SA

Concerning the second application, the determination of salicylic acid was performed by DPV and chronoamperometry, in 0.1 M NaOH. The techniques exhibited different performances. With DPV the linearity range extended from 1×10^{-5} to 5×10^{-4} M, with a LOD of 6×10^{-6} M. Differently, with chronoamperometry the linearity range was between 5×10^{-7} and 1×10^{-4} M, with a LOD of 2×10^{-7} M. The modified electrode was employed for the determination of salicyilic acid in BAYER Aspirina® samples.

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O20

ENVIRONMENTAL FRIENDLY SYNTHESIS OF POLYMER-STABILIZED METAL NANOPARTICLES AS HIGHLY SENSITIVE AND SELECTIVE SENSING PLATFORM FOR THE DETERMINATION OF CAFFEIC ACID

Gabriella Di Carlo[1], Roberta G. Toro[1], Gabriel Maria Ingo[1], Giuseppina Padeletti[1], Daniela Zane[2], Chiara Bianchini[2] and Antonella Curulli [2],

[1]CNR Istituto per lo Studio dei Materiali Nanostrutturati (ISMN) UOS Montelibretti
Via Salaria Km 29,300 00015 Monterotondo Stazione (Roma) - Italy
[2]CNR Istituto per lo Studio dei Materiali Nanostrutturati (ISMN) UOS Sapienza
Via del Castro Laurenziano 7 00161 Roma
email: antonella.curulli@ismn.cnr.it

The development of sensitive and selective methods for the detection of antioxidant agents, as polyphenols, has received much attention, due to their benefits on human ageing process and their potential pharmacological applications, e.g. for cancer and neurodegenerative diseases. In particular, the caffeic acid, a phenolic acid deriving from cinnamic acid, has been found to be pharmacologically active as antioxidant, antimutagenic and anticarcinogenic agent [1-3]. For these reasons, the determination of caffeic acid in low level and in complex matrices, such as orange juice, coffee or wine, without interference from ascorbic acid or other hydroxycynnamic acids is an important challenge. To this purpose, the attention has been recently devoted to the development of electrochemical procedures [4], which allow achieving higher sensitivity, selectivity and rapid response time with respect to conventional analytical methodologies [4-7].

Herein, we present the first study on the design of polymer-metal nanoparticles (MeNps) nanocomposites as highly sensitive, selective and rapid response time electrochemical sensors for the determination of caffeic acid. The selective responses towards our target molecule, also in the presence of other antioxidants such as ascorbic acid, make these materials of great interest for the analysis of complex matrices.

Moreover, we have demonstrated that it possible to tune the electrochemical properties of polymer-stabilized MeNPs by using different organic acids, which can participate to metal precursor reduction and to a certain extent to the stabilization of the resulting metal nanoparticles. The role played by carboxylic acids in the synthesis of polymer-MeNPs has not been investigated so far

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O21

ELECTROSYNTHESIZED POLYPYRROLE ON MICROSTRUCTURED SILICON: TECHNOLOGY, CHARACTERIZATION AND PRELIMINARY SENSING APPLICATIONS

E. Mazzotta[1], C. Malitesta[1], S. Surdo[2], L. M. Strambini[2], G. Barillaro[2]
[1] *Dipartimento di Scienza dei Materiali, Università del Salento, via Monteroni 73100 Lecce*
[2] *Dipartimento di Ingegneria dell'Informazione: Elettronica, Informatica, Telecomunicazioni, Università di Pisa, via G. Caruso 16 56126 Pisa*
elisabetta.mazzotta@unisalento.it

Polypyrrole (PPY) is an electroactive conducting polymer with application in various research and commercial fields, including sensors [1], actuators [2], corrosion protection [3] and photovoltaic devices [4]. The good electrical conductivity, the doping and dedoping process, and the easy and rapid synthetic scheme of PPY justify its large use as electrode coating [1]. In particular, the electrochemical synthesis represents an effective way of easily interfacing PPY with the electrode surface allowing the deposition of films with controlled thickness even on complex geometry surfaces [5]. Recently, research has been devoted to the miniaturization of electrosynthesized PPY-based devices [6, 7] with the aim to construct complex micrometer- and nanometer-scale systems offering advantages in view of their larger surface area and, in turn, of the higher rate of interface processes. A polymeric thin film having both high conductivity and fine structure at the micro- or nanoscale is a suitable material particularly as sensing element in the fabrication of sensor devices [6, 7]. Various methods for preparing micro- and nanostructured PPY films have been proposed, which are generally based on template-assisted synthesis [7] exploiting carbon nanotubes [8], porous alumina templates [9], and porous silicon [10]. A drawback of this method is that dimension and morphology of the PPY structures is limited by the template architecture. Moreover, in some cases, PPY electrosynthesis requires a preliminary modification of the template surface to make it conductive [9]. Finally, each polymerization step needs a single-use template that is removed after film deposition, typically by chemical etching.

The present work describes a novel approach for the development of microstructured PPY films. The proposed approach is based on PPY electrosynthesis on microstructured silicon substrates prepared by electrochemical micromachining [11,12]. Electrochemical micromachining is a low-cost high-flexible technique allowing for silicon microstructuring at the microscale [13]. The great flexibility of silicon micromachining techniques for the fabrication of three-dimensional microstructured systems is here conjugated, for the first time, with conducting polymers technologies, thus leading to the development of novel PPY films with three-dimensional features that can be selected on the basis of specific applications. Experimental conditions for PPY electrosynthesis on silicon substrates have been firstly selected and different thickness films have been prepared. The influence of silicon microstructure has been tested by performing PPY electrosynthesis, under the same experimental conditions, on flat substrates and on silicon substrates integrating regular array of square-like pores with pitch of 8 □ m, size of 5 □ m and depth of 5 □ m and 10 □ m. Interestingly, Scanning Electron Microscopy (SEM) analysis revealed that a three-dimensional polymer structure perfectly replicating the silicon microstructure is achieved on micromachined substrates. An isotropic PPY growth, i.e. same growth rate both in the horizontal and vertical direction, occurs, thus resulting in a constant thickness PPY layer uniformly covering the microstructured silicon surface. Evaluation of the film thickness by SEM analysis also allowed the correlation with the circulated charge during PPY electrosynthesis to be established. PPY films have been characterized also by X-Ray Photoelectron Spectroscopy (XPS) to check the degree of polymer overoxidation. Sensing properties of microstructured PPY have been evaluated by analyzing its amperometric responses to target analytes (dopamine and ascorbic acid). Preliminary evidences on the role of micrometer scale morphology in enhancing film recognition properties have been gained.

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O22

DAI FILM DI Hg AI FILM DI Sb : VERSO LA SOSTITUZIONE DEL MERCURIO IN ELETTROANALISI INORGANICA

Carlo Dossi, Simone Farina, Fabrizio Fusi
Dipartimento di Scienze Chimiche ed Ambientali,
Università dell'Insubria
Via Valleggio, 11 22100 COMO
e-mail : carlo.dossi@uninsubria.it

La sostituzione del mercurio metallico in elettroanalisi inorganica è un argomento da lungo dibattuto, per le evidenti implicazioni tecnologiche ed ambientali.
I nanofilm di mercurio supportati sono una alternativa spesso efficiente, anche se richiedono un'attenta scelta del legante in condizioni catodiche di stripping adsorbitivo. Non risolvono, tuttavia, i requisiti di una completa rimozione del mercurio.
Il bismuto presenta invece evidenti vantaggi in termini di ecocompatibilità e tossicità, anche se soffre di potenziali problemi di limitazioni cinetiche in riduzione e in condizioni di stripping a basse concentrazioni, vicini al LOD.
Più recentemente, inizia ad affermarsi anche l'antimonio come potenziale sostituto del mercurio in stripping elettrochimico. Non soffre certamente di limitazioni cinetiche in riduzione, ma l'expertise sembra ancora limitata, e i dati presenti in letteratura ancora scarsi.

In questa comunicazione, si affronteranno in maniera interdisciplinare le problematiche di preparazione ed utilizzo di film metallici per l'analisi inorganica in ultratraccia in condizioni di stripping elettrochimico. Per quanto riguarda i film di mercurio, si valuterà l'efficacia e l'usabilità di leganti polidentati in condizioni di stripping per adsorbimento. Nel caso, invece, di Bi ed Sb, si analizzeranno i parametri cimico/fisici per la preparazione del nanofilm, cercando di studiare ed ovviare limitazioni cinetiche e LOD/LOQ.

O23

NOVEL ELECTRODE MATERIALS FOR AMPEROMETRIC DETECTION OF OXIDANTS

Fabio Terzi[1], Barbara Zanfrognini[1], Laura Pigani[1], Chiara Zanardi[1], Renato Seeber[1], Antti Viinikanoja[2], Jukka Lukkari[2]

[1] Dipartimento di Chimica, Università degli Studi di Modena e Reggio Emilia, Via G. Campi 183, 41125, Modena (Italy) fabio.terzi@unimore.it

[2] Department of Chemistry, University of Turku, FIN-20014 Turku (Finland)

The determination of oxidising species in drinking and waste water is crucial in order to achieve a reliable process control in a large number of industrial sectors; when their concentration in water bodies exceeds certain level, they are considered harmful for humans and wildlife, as testified by national and international regulations. Different detection systems have been developed so far, the spectrophotometric methods being most popular. At the moment, methods that are reliable, selective and simple at the same time are still absent. In this frame, the development of amperometric sensors for oxidising species represents a significant innovation: similar systems are easy to use, cheap and potentially portable. They can also be employed for online measurements, as well as detectors in chromatographic systems. Au, Pt, Hg and carbon-based electrodes have shown to constitute interesting sensing probes for these species. However, these materials lack of selectivity, being unsuitable to distinguish among the different oxidising species present in the samples at the same time. In addition, the presence of interfering species, such as oxygen, prevents from application to real matrices.

In the present contribution, the development of novel electrode materials is reported. In particular, Pt electrodes have been modified by a thin layer of a conducting polymer, namely poly(3,4-ethylenedioxythiophene) – PEDOT. The modified electrode exhibits a low limit of detection, good sensitivity, reproducibility and repeatability in the determination of monochloroamine, as testified by voltammetric curves and calibration plots obtained by a rotating disk electrode. The presence of organic chloramines and of different interfering species does not affect the response of the systems. Significant improvements in the performance of the modified electrode are obtained by using composite materials: in particular, the addition of differently encapsulated Au nanoparticles to PEDOT drastically lowers the detection limit.

Graphene-based materials constitute a novel, potentially powerful family of electrode modifiers, suitable for the amperometric determination of a number of different species. Soluble oxidised graphene “aggregates” have been anchored on Au electrodes through a thiol molecule. The resulting modified electrodes exhibit promising performance in the electroreduction of hydrogen peroxide. In particular, a significant shift of the reduction potential towards less negative values has been observed, indicating the activation of electrocatalytic charge transfer processes.

O24

CARBON BLACK MODIFIED SCREEN PRINTED ELECTRODE

Fabiana Arduini[1], Fabio Di Nardo[1], Elena Suprun[2], Laura Micheli[1], Aziz Amine[3], Danila Moscone[1], Giuseppe Palleschi[1]

[1] *Dipartimento di Scienze e Tecnologie Chimiche, Università di Roma Tor Vergata, 00133 Rome, Italy*
fabiana.arduini@uniroma2.it

[2] *Institute of Biomedical Chemistry, Russian Academy of Medical Sciences, Pogodinskaya Street, 10, Moscow, Russia*

[3] *Faculté de Sciences et Techniques, B.P. 146, Mohammadia, Morocco*

In the last years a wide variety of electrode materials, and especially nanostructured materials, was explored to improve analytical parameters of electrochemical sensors in terms of sensitivity, selectivity, stability etc. Focussing on carbon materials, the Compton group highlighted that the superior electrocatalytic properties of carbon nanotubes can be attributed to edge-plane-like defects which are present at the open ends of nanotubes [1]. This interesting work encouraged us to investigate a low cost material such as carbon black (CB), which is a nanomaterial characterised by a large number of defect sites. In this work the commercial CB N220, furnace-produced type, was chosen because we have previously demonstrated [2], by means of Raman spectra, its high number of disorder and defect sites, comparable to literature values reported for carbon nanotubes [3]. In order to develop a CB sensor characterised by good reproducibility, commercial SPEs were modified with CB dispersion prepared in acetonitrile by "film" deposition. The CB-SPEs showed an enhanced oxidation current for several analytes such as NADH, cysteine, thiocholine and also the reduction of the peak-to-peak separation in the case of epinephrine, norepinephrine and benzoquinone, when compared with the bare SPE [2]. In the present work, the SPEs produced in our laboratory were modified *via* bulk or "film" deposition. The sensors prepared with different percentage of CB (5% and 10% w/w in graphite ink) and by "film" deposition using a CB dispersion prepared in DMF/H_2O were electrochemically and morphologically characterised. The "film" modified CB-SPE showed the highest amount of CB present on the surface of working electrode in SEM images, which allowed better electrochemical performances as demonstrated by cyclic voltammetry experiments using potassium ferricyanide as probe. The film CB-SPE was tested using cyclic voltammetry technique with several potentially interesting analytes such as NADH, H_2O_2, epinephrine and benzoquinone, observing, also in this case, a decreased oxidative potential (i.e. NADH and H_2O_2) or reduced the peak-to-peak separation (i.e. epinephrine and benzoquinone) when compared with the bare SPE. Using the Nicholson method [3] and considering α equal to 0.5 (owing to the fact that Ipa/Ipc value is very close to unity) we have calculated the heterogeneous rate constant (k^0) for CB-SPE and potassium ferricyanide, which resulted equal to $k^0=(2.0\pm0.2)\times 10^{-2}$ cm/s, value in agreement with our previous value obtained (1.59×10^{-2} cm/s) using a commercial available SPE modified with a CB dispersion prepared in acetonitrile. CB bulk modified SPE were also produced and tested, together to the film CB-SPE, with some heme proteins such as cytochrome *c (*cyt *c)*, myoglobin (Mb), horseradish peroxidise (HRP) and cytochrome P450 (CYP 51). The proteins were immobilized on the electrode surface by using DDAB (didodecyldimethyl ammonium bromide) or tested in solution on DDAB-CB-SPE. The modification of electrodes with CB significantly improved the electron transfer between the electrode surface and the protein active heme–centre: Fe (III) + e → Fe (II). Compared with carbon nanotube modified SPEs, the baseline current of CB-SPEs was lower and the signal/noise ratio higher. The combination of CB and DDAB for heme proteins detection and immobilization is a very promising way for obtaining new "third generation" biosensors.

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POSTER

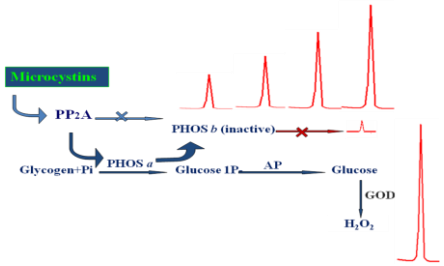
P1

A BI-ENZYME ELECTROCHEMICAL PROBE FOR FLOW INJECTION ANALYSIS OF CYANOBACTERIAL HEPATOTOXINS BASED ON PP2A INHIBITION

G. Volpe, G. Di Pofi, D. Moscone, G. Palleschi

Dipartimento di Scienze e Tecnologie Chimiche, Università di Roma Tor Vergata, Via della Ricerca Scientifica 1, 00133 Roma, giulia.volpe@uniroma2.it

Around the world, numerous wildlife and livestock deaths have been attributed to blooms of fresh water cyanobacteria (blue green algae). Cyanobacteria, in fact, can produce toxins belonging to various chemical classes. Over 60 toxins have been now recognized, including neurotoxins, hepatotoxins, cytotoxins and lipopolysaccharide (LPS) endotoxins. The hepatotoxins comprise cyclic heptapeptides (microcystins), produced by *Microcystis, Oscillatoria*, *Anabaena and Planktothrix* and a pentapeptide (nodularin) produced by *Nodularia*. Cyanobacteria and their toxins, especially microcystins, represent a drinking water public health issue. In order to assure the water quality and the public health, the WHO (World Health Organization) has recommended a maximum level of 1 µg/mL of microcystin-LR in drinking water. Rapid and reliable analytical methods capable of determining microcystins in water at concentrations ≤ of 1 µg/mL are therefore required. A promising approach in measuring microcystins and nodularin is based on their mechanism of action; they are, in fact, potent inhibitors of PP2A and PP1 enzymes. The degree of inhibition of these enzymes can therefore be used as a measure of toxin concentration.
In this work, a bi-enzyme electrochemical probe has been assembled and used to monitor the inhibition of the enzyme PP2A by microcystin-LR and nodularin. This enzyme has a significant activity towards glycogen phosphorylase *a* (PHOS*a*), which in turn catalyses the conversion of glycogen to glucose-1-phosphate (G-1-P). The proposed system involves a preliminary phase of off–line enzymatic incubations (microcystins/PP2A, PP2A/PHOS*a*, PHOS*a*/glycogen+phosphate) followed by the electrochemical detection of H_2O_2 which is the final product of two sequential reactions catalyzed by glucose oxidase (GOD) and alkaline phosphatase (AP), co-immobilized on a H_2O_2 Pt probe inserted into a FIA system. The principle of the method is the following:



The total analysis time includes 50 min for the off–line enzymatic incubations and 3 min for the biosensor response.
The system calibration shows a working range of 0.5-1.3 ppb and 5-24 ppb for nodularin and microcystin-LR, respectively. These values, referred to toxin concentrations in the final assay solution, correspond to 5-13 ppb for nodularin and 50-240 ppb for microcystin-LR in water samples.
For this reason, in order to assess the maximum level recommended for microcystin-LR, water samples have to be concentrated prior to the analysis. Preliminary results obtained analyzing *Planktothrix rubescens*-contaminated water samples, with a preconcentration step (using SPE Carbograph 4) will be presented. Experiments to improve the sensitivity of the method, to allow the direct analysis of water samples, are in progress.

P2

COPPER NANOPARTICLES/POLY-3-METHYLTHIOPHENE COMPOSITE FOR NON-ENZYMATIC GLUCOSE SENSING IN A FLOW INJECTION SYSTEM

E. Mazzotta[1], M.R. Guascito[1], C. Malitesta[1], R.A. Picca[1], T. Siciliano[2], M. Siciliano[2]
[1]Laboratorio di Chimica Analitica, [2]Laboratorio di Sensori Chimici,
Dipartimento di Scienza dei Materiali, Università del Salento, via Monteroni 73100 Lecce (Italy)
elisabetta.mazzotta@unisalento.it

The use of nanoparticles in electroanalysis is a continually expanding area of research as shown by the wealth of available research papers about the synthesis, characterization and application of nanoparticles [1]. This is due to the unique properties of nanostructured materials (e.g. enhanced mass transport, high surface area, improved signal-to-noise ratio) making their use very advantageous in many electroanalytical techniques. The advances of nanotechnology have opened up interesting research opportunities on nanocomposite fabricated not only with different nanostructured materials, but also with various conducting polymers [2]. Such composite materials have an advantage to possess properties of the individual ones with a synergistic effect [2]. In particular, the design of composite materials consisting of a mixture of organic and inorganic phases in the nanometer range has flourished in the last few years. Different strategies for fabrication of nanocomposites have been reported in literature, among which the entrapment of metal nanoparticles in conducting polymers [see: e.g. 3-5] revealed to be a simple and effective approach producing nanostructured materials with remarkable catalytic properties [6-8].
In the present work a simple non-enzymatic sensor for glucose detection has been fabricated being based on a hybrid film of electrosynthesized poly-3-methylthiophene modified by copper nanoparticles (P-3MT/CuNPs). The deposition of copper was achieved by applying a potential pulse program [9] both on Pt and on screen-printed electrodes (SPEs). The microscopic characterization of the film was performed by scanning electron microscopy/energy dispersive X-ray analysis (SEM) and showed a correlation between the pulse width and the amount and size of the deposited particles. The nanocomposite P-3MT/CuNPs was analyzed also by X-ray photoelectron spectroscopy (XPS). Performed electrochemical tests showed that P-3MT/CuNPs exhibited a remarkable electrocatalytic activity for glucose oxidation. The composite film deposited on SPEs was used for glucose detection in a flow-injection analysis system. The effect of the applied potential as well as of the flow rate of carrier stream was evaluated: under the selected experimental conditions, the film revealed a satisfactory response in terms of detection limit, linear range and repeatability. The sensitivity of P-3MT/CuNPs to other compounds (ascorbic acid, uric acid, sorbitol, fructose, dopamine) was verified evidencing that the proposed system could be effectively used as an electrochemical detector coupled to a chromatographic system for the simultaneous detection of biomolecules.

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P3

DISPERSION AND FUNCTIONALIZATION OF GRAPHENE IN IONIC LIQUIDS: TOWARDS THE ASSEMBLING OF CHEMICAL SENSORS AND BIOSENSORS.

Federica Valentini[1], Davide Roscioli[1], Marilena Carbone[1], Valeria Conte[1] , Barbara Floris[1] and Giuseppe Palleschi[1]

[1] *Dipartimento di Scienze e Tecnologie Chimiche, Università Tor Vergata, via della Ricerca Scientifica 1, 00133 Rome*

e-mail: federica.valentini@uniroma2.it

Following early attempts for producing graphene by mechanical exfoliation of highly oriented pyrolitic graphite (HOPG), many research groups are seeking high-throughput processing routes. In this paper, we propose the use of ionic liquids (salts with organic cations , whose melting point is below 100 °C) to obtain stable and homogeneous nano-emulsions of graphene nanoribbons and to perform electrochemical synthesis of graphene nanomaterials based on an ILs-assisted electrochemical exfoliation of graphite electrode. Most importantly, ILs have surface tensions very close to the surface energy of graphite, which is a solvent key prerequisite for direct exfoliation of graphite. In addition, the basic structural attribute of ILs (their ionicity), appears to be a unique feature for stabilization of exfoliated graphene via Coulombic interactions. Such advantages over most solvents make ILs the ideal systems for synthesis of graphene. In the present study, a characterization of the new graphene based nanomaterials has been carried out under a topographic (by SEM/EDX) and structural (by FT-IR, UV-Visible and XPS) point of view. Subsequently, an electrochemical investigation has been performed and the best electrochemical results were observed by using IL: H_2O mixtures 50 : 50 (% w/w) with [$BMIM^+$[Cl^-] (1-butyl -3- methylimidazolium chloride) and [$Bupy^+$][Cl^-] (1-butylpyridinium chloride), to obtain stable dispersions of oxidized graphene nanoribbons prepared as described in our previous paper[1]. NADH (for biosensors application), dopamine and serotonin (two important neurotransmitters involved in Parkinson's and Alzheimer's diseases), ascorbic acid, caffeic acid (for alkaloids detection) have been successfully detected by using graphene/IL modified SPEs (Screen Printed Electrodes; ⌀=3mm), working in a new drop detection mode. The observed enhancement of the electro-analytical current signal and the potential shift at lower values, could be explained by the presence of oxygenated functional groups on the graphene surfaces, that contribute to catalyze the electron transfer based mechanism for these molecules. In the second case, using functionalized multi-layers of graphene gel, obtained by the electrochemical exfoliation performed in 90 : 10 % w/w of [$BMIM^+$[Cl^-] and H_2O, the electro-active probes, $Fe(CN_6)^{3-}$ and $Ru(NH_3)^{3+}$ have been successfully detected, assembling a graphene/IL gel-like modified carbon paste electrodes. Further electro-analytical investigations will be necessary to understand the graphene based sensor performances and to highlight the kinetic of the electron-transfer reaction, in presence of: 1. several different % of the IL in the modified gel-like carbon paste electrodes; 2.several different ILs, used for the electrochemical exfoliation of graphite electrodes.

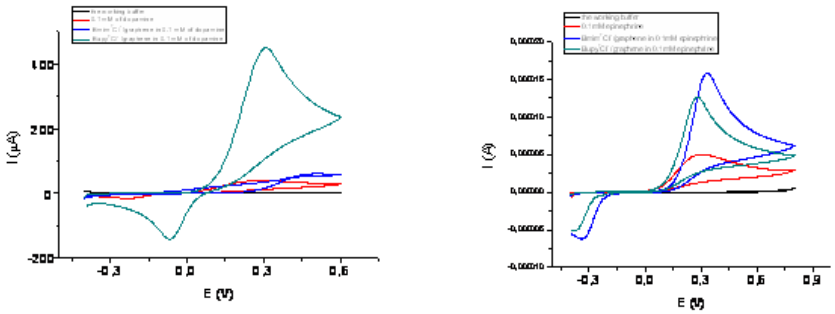

Figura 1. (**a**): Cyclic Voltammetry (CV) of 0.1mM of dopamine, recorded at graphene/IL modified SPEs; (**b**):the same but for 0.1mM of epinephrine.

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P4

DISPOSABLE SENSORS TO CONTROL TRACE METAL IONIZATION USED TO TREAT PATHOGENS IN WATER DISTRIBUTION SYSTEMS

Diego Voccia, Serena Laschi, Ilaria Palchetti, Giovanna Marrazza, Marco Mascini
Dipartimento di Chimica, Università degli Studi di Firenze, ilaria.palchetti@unifi.it

The control of hazardous pathogens in water distribution systems, is a priority for health authorities world wide. An estimated 8,000 to 18,000 people get Legionnaires' disease in the United States each year. Hospitals, hotels, old people's homes, prisons and ships are high risk environments due to the nature of the water distribution system. Treatment is essential, and one of the most effective methods is copper-silver ionization. The positively charged copper and silver ions thus released, form electrostatic bonds with negatively charged sites on bacterial cell walls; this leads to cell lysis and cell death. Importantly, some authors have demonstrated that these ions are able to penetrate the biofilms in which other bacteria, algae, protozoans, and fungi cohabit with Legionella species in water pipes. The amount of copper and silver must remain within a certain range for efficiency, and at the same time remain well below the WHO and other guidelines. High oral intake of copper and silver can result in liver failure and argyria (blue-bluish grey discoloration of the skin) for copper and silver respectively. Recommended values for copper are between 0.3 and 0.5 mg l-1 and, for silver, between 0.03 and 0.05 mg l-1.

The specific aim of this work was to study the electrochemical behaviour of screen-printed graphite electrodes in the determination of silver and copper, with the final purpose of development and construction of mercury-free electrodes to be used in the determination of silver and copper concentrations in water samples by anodic stripping voltammetry.

Particular attention was focused on the chemistry of complex formation in solution optimizing pH and reagents concentration to obtain the better reproducibility, dynamic range and selectivity.

P5

ELECTROCHEMISTRY AND ELECTROCHEMICAL DETERMINATION OF OLANZAPINE AND RISPERIDONE AT GOLD ELECTRODE MODIFIED WITH CARBON NANOTUBE.

Daniele Merli, Daniele Dondi, Antonella Profumo, Maria Pesavento
Dipartimento di Chimica, Università degli Studi di Pavia, Via Taramelli 12, 27100 Pavia
daniele.merli@unipv.it

Atypical antipsychotics are a widely used class of neuroleptics which have reached in recent years the top-sell categories of pharmaceuticals, due to their peculiar characteristics.
Owing to their widespread use, rapid, efficient and economical methods of analysis of these compounds in pharmaceutical formulations and biological fluids are required and, in this sense, electrochemical techniques appear particularly attractive. In this study, we have explored the electrochemistry of olanzapine and risperidone, two of the most used atypical antipsychotics, and an analytical method for their determination is presented.
These compounds are reported to be electroactive but quantification at classic electrodes is unsuitable due to fouling phenomena and a very poor linearity range between concentration and current. To overcame these problems, we have assembled a chemically modified gold electrode based on oxidized single-walled carbon nanotubes, that are known to facilitate the electron transfer with electroactive species in solutions and thus to alleviate surface fouling effects[1]. This chemically modified electrode was useful for the determination of the two antipsychotics and the common excipients present in their formulations (i.e. starch, sucrose and arabic gum) do not change their peak height or profile.
A deep insight in the electrochemistry of these compounds is reported, with the aid of mass spectrometric techniques and DFT (Density Functional Theory) calculation; in particular, a computational approach of their electrochemical behavior, scarcely used in this field, was proving to be of great concern.

Riferimenti

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P6

GOLD NANOPARTICLES LAYER ON GOLD CDTRODE: A PROMISING PLATFORM FOR AMPLIFIED ELECTROCHEMICAL DETECTION OF PESTICIDES

Glauco Pilon dos Santos[1], Giuseppe Christian Fusella[2], Petr Skladal[3], Marcello Mascini[2], Hideko Yamanaka[1], Dario Compagnone[2]

[1] *Department of Analytical Chemistry, São Paulo State University (UNESP), Rua Prof. Francisco Degni, s/n – 14800-900 – Araraquara/SP, Brazil, glauco@iq.unesp.br*

[2] *Department of Food Science, University of Teramo, Teramo, Italy*

[3] *Department of Biochemistry, Masaryk University, Brno, Czech Republic*

The investigations on nanotechnology are increasing, and the use of gold nanoparticles has been received considerable attention due to their potential application for developing biosensing devices. The increasing of the area/volume relationship and consequently the attached biocomponent on the sensing surface improves the biosensor response. In addition, gold surface can be easily modified by thiol ended molecules, which makes then suitable for many different biological assemblies. The aim of this study consists on the deposition of a gold nanoparticles layer on gold CDtrode (Au-CDtrode), in order to improve the sensitivity of the biomimetic sensor for the determination of pesticides (carbamate and organophosphate) in food samples. The Au-CDtrodes, electrodes constructed from gold recordable compact discs (CD-R)[1], have shown an electrochemical performance comparable with commercial gold electrodes, presenting many advantages as simplicity of construction, low cost and if necessary, disposable.

Usually the biological materials are unstable and present a high production cost. Therefore, the design and development of artificial oligopeptides as a mimic of the acethylcholinesterase (AChE) binding site, preserving the highly selective biological properties[2], was the approach used in this work. The oligopeptide proposed by molecular modelling was synthesized by solid phase peptide synthesis methodology, being immobilized on gold surface by the thiol group from the cysteine residue. Gold nanoparticles in different sizes were synthesized using sodium borohydride or sodium citrate as the reduction agent. The first method consists on the addition of 0,01% of $NaBH_4$ in a solution containing 10^{-4} mol L^{-1} of chloroauric acid and for the another one 0,01% of $Na_3C_6H_5O_7$ was added to a boiling solution containing 10^{-4} mol L^{-1} of chloroauric acid. Nanoparticles were characterized by transmission electron microscopy and presented an average diameter of 3 nm and 15 nm by using $NaBH_4$ and $Na_3C_6H_5O_7$, respectively. The Au-CDtrode was previously submitted to a voltammetric pretreatment of 10 cycles in 0.5 mol L^{-1} sulfuric acid solution in the potential range from +0.2 to +1.5 V at a scan rate of 100 mV s^{-1}. Electrochemical measurements were carried out in a potentiostat/galvanostat AUTOLAB employing a conventional cell: Au-CDtrode (A_{geom} = 0.071 cm^2), Ag|AgCl|$KCl_{(sat)}$, Pt wire as working, reference and auxiliary electrodes, respectively. After the pretreatment, gold nanoparticles were adsorbed on Au-CDtrode and then the surface was modified with 8×10^{-4} mol L^{-1} oligopeptide by incubation for 1h at 25 ºC. As demonstrated in a previous work[3], the interaction between peptide and pesticide can be monitored by the peptide signal at -1.2 V. The current was measured by square wave voltammetry, with f=100 Hz, a=50 mV and ΔE_s=2 mV, using 0.1 mol L^{-1} $NaClO_4$ as supporting electrolyte. The peptide presented a current peak of 6 μA, 10 μA and 12 μA for the bare electrode, electrode modified with the gold nanoparticles obtained by $NaBH_4$ and $Na_3C_6H_5O_7$ method, respectively. These results present a promising tool to amplify the electrochemical detection on the development of a biomimetic sensor for pesticides. FAPESP (2010/04663-6), IRSES (230849).

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P7

GOLD NANOPARTICLES-BASED DNA ASSAY FOR THE DETECTION OF BENZO[A]PYRENE OXIDATION PRODUCTS

Dario Compagnone[1], Michele Del Carlo[1], Valentina Lanzone[1], Loris Ferretti[1], Manuel Sergi[1],Camilla Montesano[2]

[1]Department of Food Sciences, University of Teramo, via Lerici 1,64023 Mosciano S.A.,Teramo

[2]Department of Chemistry, University of Rome "La Sapienza", P.le Aldo Moro 5, I-00185, Rome

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous contaminants. Their toxic effect occurs via their activation to more reactive species, usually oxidized species, via biochemical pathways. Benzo[a]pyrene is generally considered as one of the most toxic PAHs. In this work we propose the use of gold nanoparticles (GNPs) for the detection of oxidized form of Benzo[a]pyrene via DNA based sensing. A specifically sequence of DNA (probe) was designed, for detection of DNA adducts formation. The assay is based on inhibition of the hybridization reaction between two complementary sequences following the formation of a stable adduct of DNA and submicromolar concentrations of DE-BAP. Furthermore, we exploited the unique properties of nanoparticulate materials, like high active surface area, improved selectivity and sensitivity, by the selective immobilization of the thiolated DNA probes on gold nanoparticles (affinity modules). An enzyme-amplified detection scheme, based on the coupling of a streptavidin-alkaline phosphatase conjugate and biotinylated target sequence was applied. We used two reaction patterns: in the first one, the hybridization reaction occurs in a vial (homogeneous approach) while in the heterogeneous system we immobilized the affinity modules onto screen printed carbon electrodes (SPCE) through simple deposition on electrode surface and electrochemical detection by means of differential pulse voltammetry. For the homogeneous system spectrophotometric detection was also carried out using the same reaction system and 4-nitrophenyl-phosphate as substrate. This assay appears suitable to detect all the compounds forming stable adducts with DNA and, therefore, could be used as a screening test for genotoxicity of PAHs, their oxidation products and other synthetic compounds.

P8

MINIATURASED ELECTROCHEMICAL SENSORS FOR THE DETERMINATION OF ANTIRETROVIRAL DRUG

A. M. Esteva, J. J. Piña, A. I. Balbin, A. I. Pérez, D. Compagnone* & M. Mascini*
Department of Analytical Chemistry, University of Havana, Cuba.
[*]Department of Food Science, University of Teramo, 64023 Teramo, Italy
corresponding author: anam@fq.uh.cu

Aciclovir is a synthetic nucleoside anologue to guanine, is a potent antiviral drug that has shown activity against herpes viruses, including Epstein–Barr virus and cytomegalovirus. The activity is due to intracellular conversion of aciclovir by viral thymidine kinase or possibly by cellular deoxyguanosine kinase to aciclovir monophosphate with subsequent cellular conversion to the diphosphate and active triphosphate. Aciclovir triphosphate inhibits viral DNA synthesis by inhibiting the viral DNA polymerase enzyme as well as being incorporated into the viral DNA (1). As alternative to traditional chromatographic techniques for the detection of aciclovir in routine analysis we propose the use of an electroanalytical technique coupled with disposable screen-printed electrodes, using a portable potentiostat (www.palmsens.com). (2). There are few electrochemical studies of acyclovir. For this work we used cyclic voltammetry employing different modification of screen printed electrode, using as working electrodes: gold, mercury film on graphite, and Graphite. In addition to this we evaluated the response of the working electrodes to aciclovir, using two media: Briton-Robinson (BR) 0.04 M pH=2 and Phosphate Buffer Solution (PBS) 0.05M pH =6.7. In all cases there was a well-defined analytical signal as a function of the working electrode employed. The response of the scan rate on the reduction wave obtained using the gold electrode was: log Ip = 1.42 +0.4logVb R^2 = 0.94, which does not correspond to a purely diffusion. The entire analysis time was shorter than 2 min. No pre-treatment was required. In all cases the concentration was of 26 ppm.

Electrode surface	Electrolyte	Signal (mV)
Gold	PBS 0.05M pH =6.7	P_{red} -870
Graphite	BR 0.04 M pH=2	P_{Ox} 100
graphite Modified with Hg	PBS 0.05M pH =6.7	P_{red} -40 P_{Ox} 80

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P9

MODIFIED SCREEN-PRINTED ELECTRODES USING OXIDIZED GRAPHENE NANORIBBONS: AN ELECTROCHEMICAL CHARACTERIZATION STUDY IN PRESENCE OF SEVERAL BIO-MOLECULES.

Federica Valentini[1], Daniela Romanazzo[1], Marilena Carbone[1], and G. Palleschi[1]
[1] *Dipartimento di Scienze e Tecnologie Chimiche, Università degli Studi di Roma Tor Vergata, via della Ricerca Scientifica 1, 00133 Roma,*
e-mail: federica.valentini@uniroma2.it

In this paper, an electrochemical study of several molecules has been carried out, assembling graphene modified Screen Printed Electrodes (SPE) and optimizing several electro-analytical parameters. Tests were performed with different solvents to obtain a stable dispersion of oxidized graphene nanoribbons, the best nanoribbons concentration for a homogeneous coating of the electrode surfaces, the scan rates and the potential window for the electrochemical selective detection of several bio-molecules. NADH measurement has been successfully performed by Differential Pulse Voltammetry (DPV) at lower potential value (i.e.: 0.440 V, vs.Ag/AgCl, compared to the literature, [1]), with a good detection limit (of 200□M), sensitivity (of 1.70 □A/mM mm^2), and good reproducibility (RSD% ranging from 3 to 20, as inter SPE electrodes reproducibility) and this is very promising to assemble biosensors and ethanol based fuel cells. In addition, an important neurotransmitter for selective detection in clinical field application for neurological disorders, such as dopamine has been successfully recorded at graphene modified SPEs in presence of a common interferences (for instance, uric acid physiological levels), showing a good detection limit (of 4μM), sensitivity (17.47 □A/mM mm^2) and reproducibility (RSD% ranging from 7 to 20, as inter SPE electrodes reproducibility). Finally, the oxidized graphene nanoribbons did not show a significant electro-catalytic effect toward the H_2O_2 (as substrate of several oxidase enzymes), according to the literature [2], where only the reduced graphene nanostructures are active towards the oxidation of the H_2O_2. Consequently, a discussion of the electro-catalytic properties (evaluated by Cyclic Voltammetry-CV, and DPV) and the electronic features (carried out by Scanning Tunneling Microscopy/coupled with the Scanning Tunneling Spectroscopy) of these new materials will be also discussed and related to the presence of structural oxygenated defects, on their surfaces and walls.

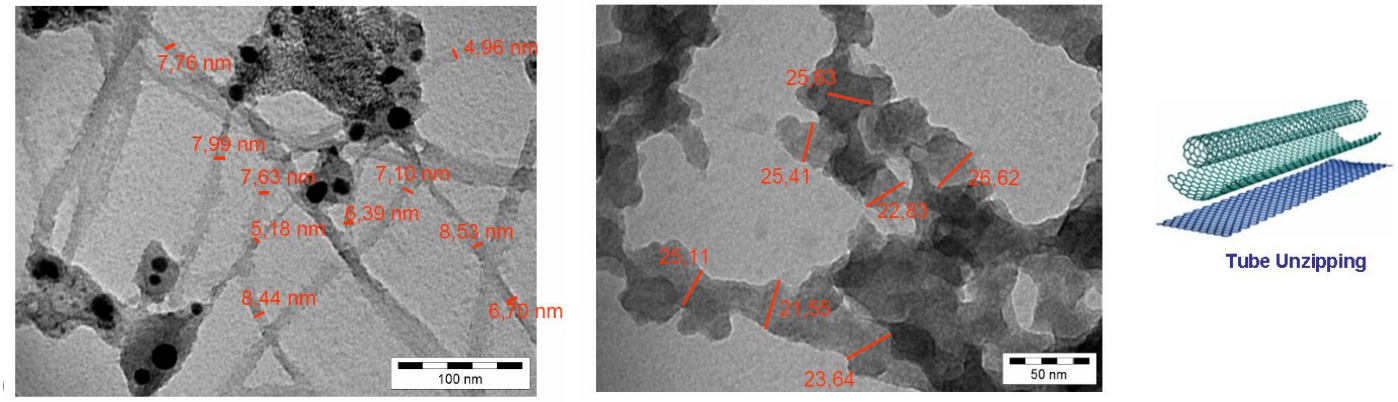

Figura 1. TEM micrographs of the (**a**): Single-Wall Carbon Nanotubes-precursor; (**b**):the synthesised graphene nanoribbons from the oxidative unzipping of SWCNTs, [F. Valentini, G. Palleschi, et al.; Carbon 48 (2010) 2596].

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P10

NEW OPTICAL AND ELECTROCHEMICAL BIOSENSORS BASED ON POLY(ARYLENEETHYNYLENE)S CARRYING AMINOACIDIC SIDE CHAINS

Claudio Lo Sterzo, Antonella Ricci, Marco Chiarini, Dario Compagnone, Michele Del Carlo, Alessia Pepe
Dipartimento di Scienze degli Alimenti, Facoltà di Agraria, Università degli Studi di Teramo, Via Carlo R. Lerici 1, 64023-Mosciano S. Angelo, (Teramo)-ITALY

Due to the great sensitivity of electronic delocalization and related opto-electronic properties, toward perturbations of the electronic density and/or mobility occurring upon interaction with analytes, the use of conjugated materials for chemosensing and biosensing has received much attention in recent years [1]. Therefore, these materials may act as efficient transducers, having the ability of converting the interaction with target analytes into observable electronic or optical signals. In addition, the receptor site linked to the conjugated backbone may be designed toward the recognition of specific analyte of interest.
The new sensing compounds we present in this simposium (Figure 1) are characterized by an highly ethynylated platform belonging to the family of poly(aryleneethynylene)s, one of the most important classes of conducting materials to be used for sensing purposes[2]. As recognition sites on the conjugated molecular backbones we have chosen aminoacids, in virtue of their ability to coordinate metal ions[3].

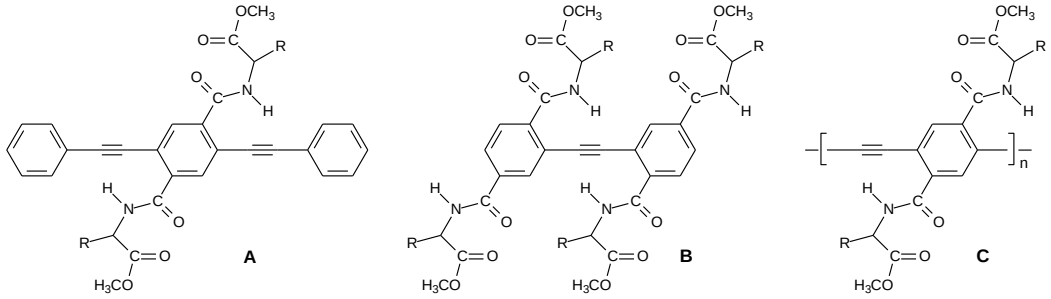

Figure 1: Chemical structure of the prepared sensing materials

The two “small molecules” **A** and **B**, bearing two or four aminoacidic units “wired” on different ethynylated platforms, were prepared to investigate the effect of different aminoacid arrangements and proximity, toward metal ion binding, while the polymeric material **C**, characterized by the regular insertion of an aminoacidc unit into a longer conjugated system, represents an example of “molecular wire approach to sensing”[4].
All these species are displaying drastic changes in the absorption and the emission spectra as well as electric response upon interaction with Hg(II). Moreover remarkable selectivity was observed, with no effect in the experimental conditions for Pb^{2+}, Cd^{2+}, Cu^{2+}, Mg^{2+}, Ni^{2+}, Mn^{2+}, Co^{2+}, Zn^{2+} at ratios M^{2+}/Hg^{2+} up to 1000:1.
Either the oligomeric and polymeric materials prepared appear very promising toward their use as sensing material for the development of sensors for the rapid assay of Hg(II).

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UN BIOSENSORE IMPEDIMETRICO BASATO SU NANOTUBI D'ORO RICOPERTI CON POLIPIRROLO OVEROSSIDATO PER LA DETERMINAZIONE DI GLUCOSIO

Chiara Bianchini[2], Antonella Curulli[1], Mauro Pasquali[2], Daniela Zane[1]
[1]*CNR-ISMN, Via del Castro Laurenziano 7, 00161 Roma*
[2]*Università degli Studi "La Sapienza" Facoltà di Ingegneria, chiara.bianchini@uniroma1.it*
Via del Castro Laurenziano 7, 00161 Roma

Il polipirrolo overossidato è un materiale ampiamente studiato per la possibilità di utilizzarlo nella costruzione di biosensori. In questo lavoro il polimero viene elettrodepositato (1,2) su un elettrodo costituito da nanotubi d'oro (AuNTs) ottenuti con una "template synthesis": una membrana di policarbonato costituisce lo stampo per la deposizione di nanofibre di Au all'interno dei pori (3,4). La deposizione del polimero sui nanotubi metallici si è resa necessaria per superare i limiti costituiti dalla piccola superficie di questi utile per l'aggancio di enzimi. Gli elettrodi costituiti dai AuNTs hanno fornito buone prestazioni per la determinazione di H_2O_2, in un range di concentrazione interessante per successive applicazioni in ambito clinico mediante l'immobilizzazione della glucosio ossidasi (GOD). Infatti in figura 1a si riportano gli spettri di impedenza registrati per concentrazioni crescenti di H_2O_2. Nell'inserto all'interno del grafico si riporta la retta di taratura che mostra la variazione del reciproco dell'R_{ct} in funzione della concentazione di H_2O_2. Il sistema costituito dai AuNTs ha dimostrato di rispondere linearmente su tutto l'intervallo di concentrazione studiato (0,063-0,317mol l^{-1}).

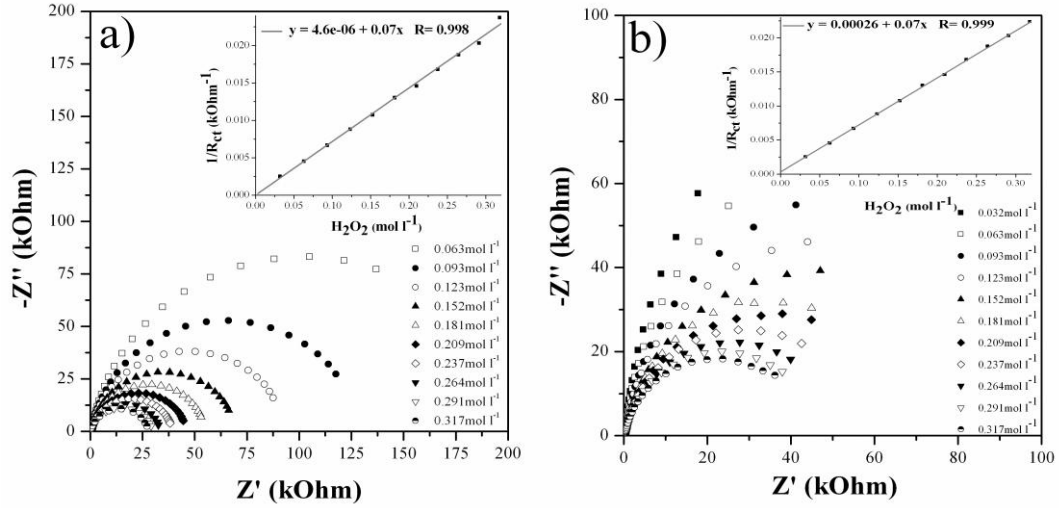

Figura 1. a) Spettri di impedenza per un elettrodo modificato con AuNTs con concentrazioni crescenti di H_2O_2 e relativa retta di taratura. Range di frequenza: 10kHz-0.1Hz. **b)** Spettri di impedenza per un elettrodo modificato con AuNTs/opPy con concentrazioni crescenti di H_2O_2 e relativa retta di taratura. Range di frequenza: 10kHz-0.1Hz.

La presenza polipirrolo overossidato (opPy) sui AuNTs non ha sostanzialmente modificato la risposta impedimetrica del sistema a concentrazioni crescenti di H_2O_2 in quanto, come dimostrato dalla figura 1b, la retta di taratura mostrata nell'inserto ha la stessa pendenza ($\Delta y/\Delta x$= 0.07) del dispositivo senza il polimero. Il sistema composito AuNTs/opPy ha dato risultati promettenti per lo sviluppo di un biosensore per la determinazione di glucosio via impedimetrica in campioni reali.

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URICASE BIOSENSOR BASED ON SCREEN-PRINTED ELECTRODE MODIFIED WITH PRUSSIAN BLUE FOR DETECTION OF URIC ACID IN HUMAN SERUM

Silvia Piermarini[1], Davide Migliorelli[1], Giulia Volpe[1], Giuseppe Palleschi[1], Renato Massoud[2], Claudio Cortese[2]
[1]*Dipartimento di Scienze e Tecnologie Chimiche, Università di Roma Tor Vergata, via della Ricerca Scientifica,00133 Roma; silvia.piermarini@uniroma2.it*
[2]*Dipartimento di Medicina Interna, Università di Roma Tor Vergata, via Montpellier 1, 00133 Roma*

The aim of this work was the development of an electrochemical biosensor for the determination of uric acid in human serum. Uric acid is the product of purine metabolism in human body and high levels in the blood are symptoms of several diseases such as gout, hyperuricemia, or Lesch-Nyhan syndrome. The typical reference range of uric acid is between 3.5 and 8.5 mg/dL.
The proposed biosensor is based on a screen-printed electrode which senses the hydrogen peroxide produced by the reaction catalysed by the enzyme uricase immobilised onto the surface of the working electrode, modified with Prussian Blue. Using a portable instrumentation (PalmSens) set on chroamperometric mode, a working range between $3x10^{-5}$ and $3x10^{-4}$ M with a response time of 30 s was obtained. The biosensor has been used to analyse up to 50 samples of human serum, without loss of activity. Recovery studies, by adding standard solutions of uric acid to human serum, were carried out. After fortification, samples were diluted 1:2 (in borate buffer 0.1 M, pH 8.5) and measured. A recovery from 92 to 105% and a relative standard deviation of ≤ 5% were obtained.
70 human serum samples, whose uric acid concentration was determined by Dimension Vista System (spectrophotometric kit), were provided by Policlinico of Tor Vergata. These samples were also analysed with uricase biosensor and the experimental data (biosensor *vs* spectrophotometric assay) showed a correlation coefficient (r^2) equal to 0.986 (Fig. 1).

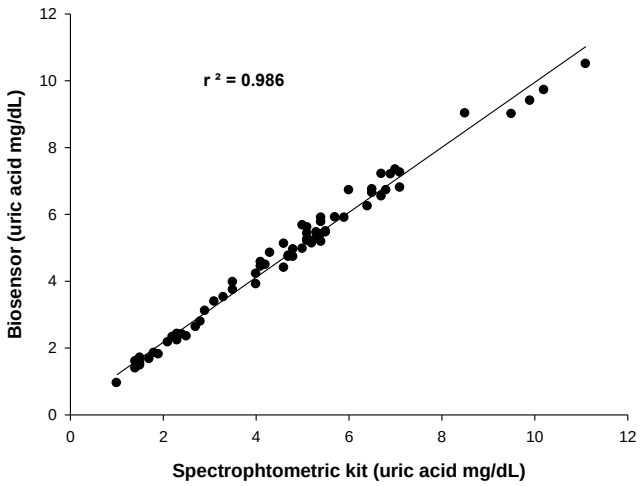

Figura 1. Correlation between uric acid values in human serum measured by biosensor and spectrophotometric kit

A more appropriate statistical method for clinical measurements, to assess the agreement between the two methods, is in progress.

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USE OF ELECTRONIC NOSE TO EVALUATE FLAVOUR CHARACTERISTICS OF CHOCOLATE SAMPLES

Pierpaolo Piccone [1], Giuseppe C. Fusella[1], Paola Pittia[1], Corrado Di Natale[2], Dario Compagnone[1]

[1] *Dipartimento di Scienze degli Alimenti, Università di Teramo,Via C.R. Lerici 1, 64023 Mosciano Sant'Angelo (TE) Italy, ppittia@unite.it*

[2]*Dipartimento di Ingegneria Elettronica, Università degli studi di Roma Tor Vergata, via del Politecnico, 00133 Roma, Italy*

Flavour properties are among the quality properties that affect food acceptability by the consumer. The peculiar aroma profile of chocolate products is related to a complex mixture of volatile compounds whom composition (nature and concentration) varies depending on both the raw materials, the ingredients used and the processing conditions applied during manufacturing. During storage, degradation reaction (e.g. oxidation, evolution of Maillar reaction) and physical processes (e.g. aroma loss) may impair the presence and concentration of the volatile components and induce changes in the aroma of the chocolate products that, in turn, may affect their acceptability. The release of an aroma compound from a food matrix is, however, affected by thermodynamic and kinetic aspects that depend not only on the chemical characteristics of the matrix, but also on environmental factors (temperature, in particular) and the interactions that may occur in the matrix between the volatile and non-volatile compounds.

In this work a preliminary study on the use of an electronic nose made by an array of eight quartz microbalances functionalized by solid state layers of metalloporphyrins (Santonico et al, 2008) was carried out to discriminate chocolate samples differently produced and/or stored based on their aroma profile. Measurements were carried out at 20°C; sensors fingerprints were analysed by Principal Component Analysis.

Results evidenced the ability of the instrument to discriminate samples with different composition or made with different process conditions (chocolate, milk chocolate). The presence of volatile compounds markers of the evolution of degradation reactions and responsible of unpleasant off-flavour was also statistically evidenced.

In conclusions, the results of this preliminary investigation confirmed the feasibility of the use of the electronic nose to evaluate and characterize the aroma profile of chocolate samples.

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VALIDATION OF A SIMPLE PROTOCOL BASED ON SW-ASV OR THE DETECTION OF PB^{2+} AND CD^{2+} IN FISH SAMPLES

Michele Del Carlo[1], Roberto Malascorta[1], Mauro Esposito[2], Pasquale Maglio[2], Federica Corrado[2] and Dario Compagnone[1]

[1]*Dipartimento di Scienze degli Alimenti, Università di Teramo*
Mosciano Stazione
[2]*Istituto Zooprofilattico Sperimentale del Mezzogiorno, Portici, Napoli*

The occurrence of Pb and Cd in fish samples remains a major problem in food safety assessment. Heavy metals are present in the aquatic environment and can accumulate in fish which is consumed as food. The poisoning from heavy metals can results in serious toxic events in humans. In 2006 the European Union has regulated the presence of heavy metals in fish and molluscs defining a maximum residue level (MRL) in the Directive 1881/2006. In the present communication we describe the validation, according to the directive 657/2002, of a quantitative screening method for Pb2+ and Cd2+ in different fish species. The analytical protocol is based on a simplified extraction protocol followed by square wave anodic stripping voltammetry using Hg° plated graphite screen printed electrodes. The protocol include a fish muscle digestion at controlled temperature in HCl followed by H2O2. The digested matrix is then filtered on a paper filter and purified on C18 SPE cartridge, previously conditioned with methanol and water. The SPE purification resulted necessary to suppress the matrix effect on the electrochemical determination. The electrochemical determination was carried out using graphite screen printed electrodes which were plated with Hg° just prior to measurement. Calibrations of the two investigates metals were carried out in the different matrix obtaining a nice linear behavior in a wide concentration range (10-500 ng/ml). Recovery was evaluate on spiked samples showing satisfactory extraction/purification results ranging between 80 and 105% at MRL. The validated method was then used to determine heavy metals concentration in fish samples previously analysed by ICP-MS, showing a nice correlation with the reference method.

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A MOS-SENSOR BASED SYSTEM TO ASSESS RESPIRATORY PHYSIOLOGY

Andrea Mazzatenta[2,5], Sergio Cozzutto[4], Pierluigi Barbieri[3], Camillo Di Giulio[2] and Nicola d'Alessandro[1]
[1] *Department of Science, University of Chieti-Pescara "G. d'Annunzio",Viale Pindaro, 42, Pescara, ITALY, dalessan@unich.it*
[2] *Physiology Unit, Department of Neuroscience and Imaging, University of Chieti-Pescara "G. d'Annunzio"*
[3]*Department of Chemical and Pharmaceutical Science, University of Trieste*
[4]*ARCO Solutions srl, Spin off Trieste University*
[5]*Department of Veterinarian Surgery Science, University of Teramo*

A biological concentration of oxygen keeps functional the cell enzymatic machinery. However, when hypoxic and oxidative stresses are established, physiological reactions induce multiple changes in the body ventilation, and the cardio-circulatory and psycho-physiological systems. A cumulative result of oxidative damage induced in cells by reactive oxygen species (ROS) derives from aerobic metabolism. Physiological antioxidant defenses maintain ROS at harmless levels preventing damage. Thus, such balance is strictly related to a constant oxygen concentration. When this balance falters, for instance in aging or chronic exposure to pollutants, a reduction in homeostatic adaptation to metabolic requirements occurs, through the activity of such enzymes as endothelial nitric oxide synthase (eNOS). Chronic hypoxia, per se, promotes a remodeling of the structure and function of the cardio-respiratory system, brain, kidney, liver, and muscles.[1]

In order to asses respiratory physiology in a non-invasive way we investigated the potential application of a new sensor system.[2,3] In healthy volunteers, who provided written informed consent and the procedures were performed in agreement with the Ethical Standards of the Helsinki Declaration, we measured the exhaled breath content in the control condition and under exposure to olfactory stressors that mimic hypoxic or pollutant stressors. The recording system used in this experiments was an iAQ-2000 (AppliedSensor) equipped with a metal oxide semiconductor (MOS) having a sensing range of 450-2000 ppm CO_2 equivalents. It is able to detect a broad range of volatile compounds (both organic and inorganic, e.g.: alcohols, aldehydes, aliphatic hydrocarbons, amines, aromatic hydrocarbons, ketones, organic acids and CO)[4] while correlating directly with CO_2 levels. A Wohler A600 gas analyzer was used to verify the O_2 and CO_2 measurements done by the iAQ-2000. The exhaled breath was collected into a face mask for the measurements. Preliminary results indicate that the recording system employed was suitable for collect physiological parameters such as breath frequency and amplitude and the analysis of the breath exhale content in humans. Interestingly, the system was able to detect and discriminate between the exhaled breath content taken from the control condition and those from conditions under stress that mimicked exposures to pollutant and/or hypoxia. Data encourage a wide application of the MOS-sensor based system in human physiology applications.

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P16

MESSA A PUNTO DI UN METODO RAPIDO PER L'INDIVIDUAZIONE DELLA CONTAMINAZIONE FUNGINA SUL CAFFE' VERDE MEDIANTE L'UTILIZZO DEL NASO ELETTRONICO

Sberveglieri V.[1], Concina I.[2], Falasconi M.[2], Gobbi E.[2], Pulvirenti A.[1], Fava P.[1]

[1] *Modena and Reggio Emilia University, Dept. of Agricultural and Food Sciences, Via Amendola, 2 - 42100 Reggio Emilia, Italy.*

[2] *SENSOR, University of Brescia & CNR-IDASC, via Valotti, 9 – 25122 Brescia, Italy*

E-mail: veronica.sberveglieri@unimore.it

Tra le problematiche di frequente riscontrate nella catena di distribuzione del caffè, lo sviluppo di muffe prima del processo di tostatura appare tra i più importanti. Questo tipo di contaminazione può portare a rischi per la salute del consumatore a causa della produzione di metaboliti tossici (micotossine)[1] e a un decadimento del prodotto andando incontro ad una perdita economica.

La contaminazione causa la presenza di sostanze derivanti dal metabolismo fungino che possono dare origine a difetti sul caffè e rischi per la salute del consumatore anche dopo la tostatura, pertanto è indispensabile mettere a punto delle strategie per individuare e quantificare precocemente un'infezione fungina e la conseguente produzione di tossine nelle prime fasi della catena alimentare. Una delle tecniche più promettenti è l'analisi dei composti volatili dello spazio di testa che circonda i campioni.

Lo scopo di questo lavoro è stato quello di verificare la capacità del naso elettronico (EN EOS [835]) di identificare rapidamente la contaminazione microbica di *Coffea arabica*.

Il nostro strumento (EN) è dotato di sei sensori di gas a base di film sottili di ossidi metallici semiconduttori ed è equipaggiato con un auto campionatore da 40 posizioni per lo spazio di testa statico.

Nel nostro lavoro i chicchi verdi sono stati precedentemente sterilizzati con raggi UV in modo da poter eliminare eventuali contaminazioni non volute e per poter essere certi di avere un controllo sterile, sono stati inoltre incubati in una camera umida a 27°C per 5 giorni per favorire la crescita dei funghi inoculati. Sono state scelte due specie appartenenti al genere *Aspergillus (A.niger* e *A.ochraceus),* capaci di colonizzare spontaneamente questo substrato. I due ceppi scelti sono stati testati in parallelo con tecniche classiche di isolamento microbiologico (per poter stimare e contare l'effettiva contaminazione nei giorni successivi all'inoculo) con tecniche classiche di tipo chimico (come il GC-MS con SPME per la rilevazione delle formazione di eventuali metaboliti secondari), mettendo in relazione quindi i risultati ottenuti con quelli acquisiti dal naso elettronico.

In particolar modo è stato quindi possibile verificare la buona abilità di questo tipo di tecnica nella valutazione precoce della possibile contaminazione del caffè verde.

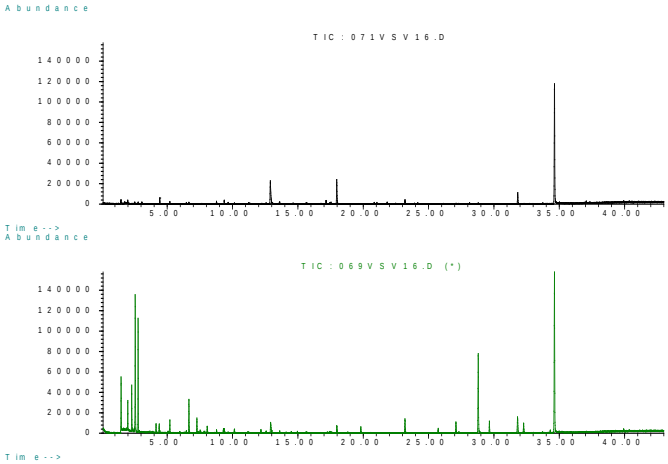

Figura1. GC-MS di caffè verde, (A) non contaminato (B) contaminato

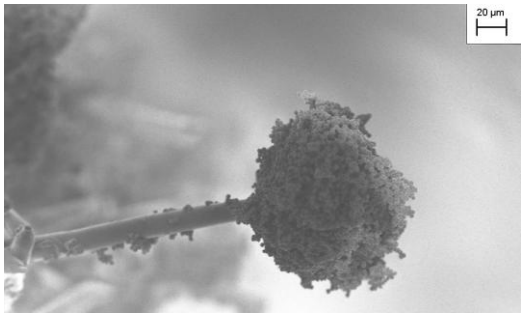

Figura 2. Presenza fungina su chicco di caffè rilevata al SEM

Riferimenti

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P17

COMPARISON OF THREE IMMUNOSENSOR METHODS (SURFACE PLASMON RESONANCE, SCREEN-PRINTED AND CLASSICAL AMPEROMETRIC IMMUNOSENSORS) FOR IMMUNOGLOBULINS G DETERMINATION IN HUMAN SERUM AND COW MILK

Mauro Tomassetti[1], Elisabetta Martini[1], Luigi Campanella[1], Luciano Carlucci[2], Gabriele Favero[3], Franco Mazzei[3]

[1]Department of Cemistry, [2] Department di Biochemistry Science "A. Rossi Fanelli", [3]Dipartimento di Chimica e Tecnologie del Farmaco,

Università di Roma "La Sapienza", P.le Aldo Moro 5, 00185, Rome,Italy

Several researches carried out by our team aimed at developing new immunological methods to determine proteins such as Immunoglobulins G in biological matrixes, such as serum and milk, tests were performed in the last years using several different immunosensors based on different types of transducers (potentiometric or amperometric electrodes) [1 – 3]; the team currently tested the feasibility of constructing immunosensors based on surface plasmon resonance (SPR) in the Kretschmann configuration. Different construction techniques and measurement procedures were used but in all cases horseradish peroxidase was used as enzymatic markers, furthermore the "competition" immunological procedures were used in two cases.

Conversely, the SPR transduction technique used in the present research allowed of a "direct" measurement procedure. The Surface Plasmon Resonance (SPR) experiments were performed using an ESPRIT instrument (Echo Chemie B.V., The Netherlands). In the experiments a sensor (Xantec Bioanalytical), consisting of a glass disk covered with a 50 nm thick Au layer superimposed on a 1.5 nm Ti layer required for the purpose of adhesion was mounted in a Teflon SPR cell. The Au surface, which was cleaned before use, was modified by dipping it into a millimolar alcohol solution of mercaptoundecanoic acid, thus obtaining a SAM (self assembled monolayer) that makes it possible to chemically bond the selected antibody (anti-IgG) to the surface by means of a reaction with carbodiimide and succinimide.

For the amperometric screen printed measurements a transducer formed on corundum ceramic was used as both working and counter electrodes were made of platinum; Pt working electrode was modified with electrodeposited Prussian Blue thus enabling H_2O_2 amperometric detection when polarized at 0 mV vs Ag/AgCl. The reference electrode was made of Silver in standard product AC1.WS.RS. At the end of the sensor there was a contacting field, which was connected to the active part by the silver conducting paths, which were covered by a dielectric protection layer. A bio-chemically active antibody (or antigen) immobilized on a Immobilon membrane was placed on the working electrode of the sensor.

The comparison of the results obtained using the electrochemical classical immunosensor developed [2] and the screen printed immunosensor [3] show that LOD for IgG determination was 10^{-11} mol L^{-1} using classical amperometric immunosensor, and 10^{-9} mol L^{-1} using the screen printed immunosensor.

Also in the case of SPR devices the LOD value was found of about 10^{-9} mol L^{-1}, but the measurement time was found to be about half of that required in two previous competition methods. Lastly SPR and classical amperometric methods were applied to the determination of IgG concentration in human serum, cow's and powdered milks obtained satisfactory results.

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