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in memoria di Marco Mascini

Sensori e biosensori: stato dell'arte e nuove prospettive

BOOK OF ABSTRACTS

Parma, 15-17 giugno 2015

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INVITED LECTURES

IL - 1

PCR-FREE DETECTION OF GENOMIC DNA BY SURFACE PLASMON RESONANCE IMAGING

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PCR amplification is a mandatory step for most of modern DNA detection assays. It requires expensive reagents and is prone to sample contamination.
Advanced methods for genomic DNA and RNA detection are requested to operate with high-specificity, multiplexed capability and ultra-sensitivity. The latter is needed in order to avoid the PCR amplification.[1]
Efforts have been recently made in identifying innovative DNA detection protocols which can be performed without PCR. Most of them exploit strategies for signal amplification based on the use of enzymes or metallic nanostructures. In particular, gold nanoparticles have been exploited to obtain an ultrasensitive DNA detection.[2]
Surface plasmon resonance imaging (SPRI) has emerged as a powerful tool to investigate interactions with biomolecules arrayed onto chemically modified metal surfaces. SPRI enables biomolecular interactions to be monitored in parallel, in real-time and without labels.[3,4]
Possibilities offered by nanoparticle-enhanced SPRI in the detection of non-amplified genomic DNA will be presented by discussing possibilities in detecting genomic DNA from soybean, bacteria and humans.[5,6]

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IL - 2

SILICON OPTICAL MICROSYSTEMS FOR LABEL-FREE BIOSENSING: THE CASE OF PHOTONIC CRYSTALS

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In the last two decades, one-dimensional (1D) photonic crystals (PhCs), as well as 2D and 3D, have been successfully used for label-free refractometric and biosensing applications [1], due to their high-sensitivity to small changes in both dielectric constant and thickness of the materials assembling the PhC structure. Delivery of liquids either over [1, 2] or through [3-5] the PhC structure has been also reported, the latter envisaging higher sensitivity and lower limit of detection than the former [3]. Pressure-driven operation, through the use of external pumps, has been commonly used for the deliver of liquids in PhCs [2, 4], which limits somehow the applications of PhCs as sensing elements for point-of-care analysis. Nevertheless, capillarity-driven operation, without the use of external pumps, has been also recently demonstrated [5], thus envisaging the development of self-powered drop-and-measure platforms based on PhCs.
Among PhC structures, vertical, high-aspect-ratio silicon/air 1D PhCs able to control light propagation in a plane parallel to the silicon substrate represent an stimulating solution for the fabrication of miniaturized platforms to be employed in (bio)sensing [6]. In fact, vertical 1D PhCs intrinsically features a microfluidic path, which is perpendicularly to and independent of the optical path and that can be used to infiltrate the liquids "trhough" the PhC structure.
Recently, deep-etching of complex silicon microstructures and microsystems with sub-micrometer accuracy at aspect-ratio values beyond standard dry and wet etching technologies has been demonstrated by electrochemical micromachining technology (ECM) [7] for both biomedical [8, 9] and photonic [10, 11] applications. ECM technology allows silicon microstructuring to overcome limitations of modern dry etching technologies, with the further advantage of the low cost of ECM technology with respect to dry etching technologies, which could make advanced silicon microstructuring available in any lab.

In this work 1D PhC all-silicon platforms, fabricated by electrochemical micromachining technology (ECM) and integrating vertical, silicon/air 1D PhCs together with fluidic and optical paths, for refractometric and biosensing applications both under pressure-driven and capillarity-driven operation are reviewed.

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Acknoledgments

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KEY NOTES

KN - 1

SMART (CELL)^{2}PHONES AS TOXICITY BIOSENTINELS

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Global security threats have become a major concern and their early detection represents a major challenge to current monitoring technologies. The routine monitoring of water, food and the environment for chemical and biological threat agents is often hampered by the fact that available techniques usually require clean samples and sophisticated equipment, and are thus unsuitable for real-time, cost-effective and on-field routine monitoring. We previously demonstrated the feasibility of implementing enzyme-based assays with bio-chemiluminescence detection into smartphones using cartridges and adaptors fabricated with user-friendly, low-cost 3D printing technology [1,2].

Here we report a portable toxicity sensor incorporating bioluminescent (BL) whole-cell biosensors into a smartphone-based device. We fabricated a 3D printed smartphone adaptor (which can be designed to fit almost any mobile device) and ready-to-use cartridges integrating an array of bioluminescent cells. We demonstrated the feasibility to accurately detect and quantify the BL signals of genetically engineered human cell lines expressing different luciferases and exploited them as a toxicity sensors using a smartphone, compared to a conventional CCD. An android app was also developed to provide a user-friendly built-it data analysis. A limit of detection, i.e., the minimum number of detectable cells, obtained by imaging Hek293T cells expressing green-emitting luciferase, of 5000 cells was obtained and toxicity test showed performance comparable to those obtained using portable cooled CCD camera, confirming the suitability of this approach. Conscious that this approach is still in its infancy and huge efforts will be required to extend the lifespan of the integrated cells without affecting the analytical performance of the system, we believe that it could find significant application as rapid alerting tool. Such validated biosensor could represent a turnkey solution for rapid, sensitive portable toxicity sensor, currently not available in the market, suitable for detecting the presence of harmful pollutants in civil and military water supplies, for terrorism surveillance, and for detection of health threats in drinking water in developing countries.

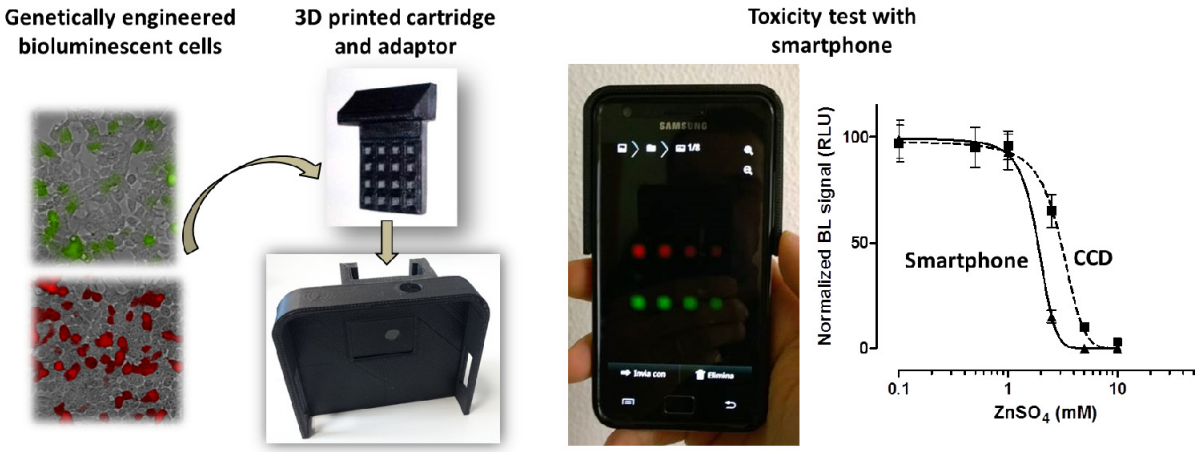

Figure 1. Overview of the SMART (CELL)^{2}PHONE device. Genetically engineered BL mammalian cells (SMART CELL) were integrated into 3D printed cartridges and exploited as toxicity biosensor using a CELL PHONE as detector.

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KN - 2

LOW COST AND SMART DIAGNOSTIC PLATFORMS FOR CHEMICAL ANALYSIS

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The fast developments in the field of consumer electronics are dramatically expanding the employment of smart, low cost devices for specific tasks like the development of biological and chemical assays. An interesting approach, particularly in the field of research, prototyping and for educational purpose is represented by the fast growing area of the universal electronic modules. These tools are widely used by schools and enthusiast for learning the basics of electronics, informatics and engineering and to make objects for entertainment, internet-of-things or accomplish specific informatics-oriented tasks. These devices are often "open source" systems, are quite inexpensive and a wide range of sensors, actuators and expansion modules are already available. The modularity, along with powerful open-source software libraries, makes them perfect tools for research, trial-and-error learning and, more in general, for cross-contamination of science fields.
Despite the great potential of such devices, very few efforts to hack and adapt these tools in life science-related fields are reported to date.
The results presented herein are focused on application of Raspberry Pi to chemistry analysis.
Raspberry pi is a low cost (30€) single-board credit-card sized computer developed with the main aim to advance the education of adults and children in the field of computers, computer science and related subject. The Raspberry Pi card and its CMOS camera, assembled with 3D printed parts and inexpensive opto-electronic components, was employed to build cheap readers for absorbance and fluorescence for food and medical analysis. The software and the user-friendly GUI that manage the whole analysis was developed entirely using Python and some free python packages. In order to test the absorbance reader for diagnostics-oriented applications, a DPPH-based assay for the evaluation of scavenging activity of antioxidant molecules was carried out using the Raspberry Pi-based device. The analysis of bottled beverages performed in triplicate was compared with a conventional laboratory-based approach showing no significant differences. The reader for fluorescent assay was employed to read fluorescein in nano-molar concentration and was able to detect positive samples of the foodborne virus norovirus after PCR amplification.
The approach presented herein is a valuable tool for low-cost, citizen involvement in science and will help the DIY approach in science, opening new opportunities in research and education.

KN - 3

SELF-CLEANING FEATURES OF AN INNOVATIVE ENGINEERED SENSOR BASED ON SILICA, SILVER NANOPARTICLES AND TITANIA

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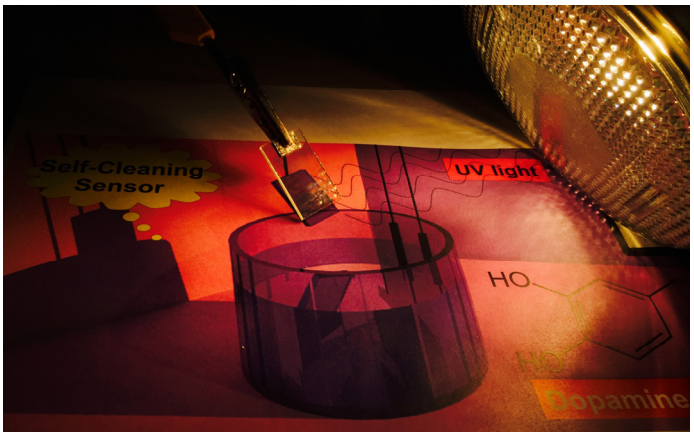
Passivation of the electrode surface and fouling are important challenges in electroanalysis during the use of modified electrodes in complex matrices, especially in the biomedical and environmental fields [1-2].
In order to overcome such problems, the production of highly engineered ad hoc designed devices could access really effective sensors [2]. In particular, a performing, reliable and reusable sensor, that could be cleaned by a simple irradiation with UV or solar light, would be perfect for this purpose.
In this context, a three-layered transparent electrode, in which silver nanoparticles are embedded between a bottom silica and a top titania layer was developed [3-4]. Such structure confers to the device multifunctional properties for a complex biomedical challenge: the detection and quantification of catecholamine neurotransmitters. The sensor was thoroughly investigated by structural, morphological and electrochemical characterizations in order to understand the role of each component with the aim to improve the robustness and efficiency of the electroanalytical system.
The overlayer was made of anatase (the active polymorph of titanium dioxide) as confirmed by X-ray diffraction and by measuring the photodegradation of model contaminants. The size distribution of silver nanoparticles, the device architecture and surface homogeneity were inspected by electron microscopy. Electrochemical techniques (cyclic voltammetry and electrochemical impedance spectroscopy) revealed that a highly ordered distribution of silver nanoparticles constitutes the active core of the device, allowing easier electron transfer and better quantification of the analytes even in the presence of conventional interferents, *e.g.* ascorbic and uric acid. Titania photoactive top layer allowed total recovery of the device performance in terms of sensitivity after a fast and simple UV-A cleaning step, affordable with different UV sources. This self-cleaning property, combined with a remarkable resistance against aging and ease of use, allows to employ the sensor also in on-field and remote applications.



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COMUNICAZIONI ORALI

O - 01

ULTRASENSITIVE AND MINIATURIZED BIOSENSOR USING ORGANICALLY MODIFIED SILICA NANOPARTICLES DOPED WITH NEW ACRIDINE-1,2-DIOXETANE DERIVATIVES AS THERMOCHEMILUMINESCENT REAGENTLESS LABELS

Massimo Di Fusco[1,2], Arianna Quintavalla[2], Marco Lombardo[2], Massimo Guardigli[2], Mara Mirasoli[1,2], Luca Alfio Andronico[2], Claudio Trombini[2], Aldo Roda[2]

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We recently demonstrated that thermochemiluminescence (TCL), i.e., the light emission originating from the thermolysis of a suitable molecule, is a powerful tool for biosensor development since it is a reagentless chemical luminescence-based detection technique, thus simplifying the microfluidic network in miniaturized analytical devices and biosensors based on the use of conventional chemiluminescence (CL). The main problems of TCL detection are the high operating temperature (200−250°C) to decompose the molecule and produce the singlet excited state, and the poorer detectability in comparison with other labels due to the low efficiency of the luminescence process.

Recently, we overcome these limitations by synthesizing a library of new TCL acridine-1,2-dioxetane derivatives proposed as new TCL labels [1-3]. Suitable structural modifications were introduced to decrease the emission triggering temperature down to 80−100 °C and to produce highly efficient fluorophores in the singlet excited state with fluorescence quantum yields (ϕ_F) ranging from 0.1 to 0.5.

To meet the detectability required for most diagnostically useful biomarkers, despite our improvements of the TCL labels with respect to the molecules reported in the past, further emission amplification is required to develop ultrasensitive immuno- or gene probe based biosensors. For this purpose organically modified silica nanoparticles (ORMOSIL NPs) doped with luminescent molecules were prepared and functionalized with biotin for binding to streptavidin-labeled species to be used as universal detection reagents for immunoassays. A quantitative non-competitive immunoassay for streptavidin was developed and the analytical performance was similar to that obtained by CL detection using horseradish peroxidase (HRP) as label [4]. In addition, since the TCL emission is simply initiated by thermolysis of the label, chemical reagents were not required, thus allowing reagentless detection with a simplification of the analytical protocols. A compact 3D-printed device based on the use of a cooled CCD and a miniaturized heater was developed and used to quantify the light emission after decomposition of the label at a temperature of 90−120 °C. These characteristics make TCL doped ORMOSIL NPs ideal universal nanoprobes for ultrasensitive bioassays such as immuno- and DNA-based assay in a compact and simple biosensor format.

More recently, we developed a smartphone-based TCL device comprising of a 3D-printed cover and a battery powered mini-heater, obtaining a further miniaturization of TCL biosensors. This device is under investigation to develop a TCL-based immunoassay for the antiepileptic drug valproic acid.

In addition, the synthesis of new TCL molecules containing different fluorophores in the backbone, i.e., fluorenone, fluorene, xanthone and flavone, or different substituents on the acridine moiety was performed to obtain more efficient TCL molecules and thus increasing the detectability of TCL-based biosensors.

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O - 02

ULTRA-SENSITIVE FLOW-THROUGH OPTOFLUIDIC MICRORESONATORS FOR BIOSENSING APPLICATIONS

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This work reports preliminary results on the design, fabrication, and preliminary characterization of three ultra-sensitive flow-through optofluidic microresonators, namely a photonic crystal resonator (Fig.1a), a bubble resonator (Fig.1b), and a ring resonator (Fig.1c), to be employed for the realization of ultra-sensitive optofluidic resonant biosensors. The microresonators are based on different operation principle, and exploit three fabrication technologies and two functionalization strategies, which are based on biological and polymeric receptors. The final objective is the exploitation of such ultra-sensitive optofluidic resonant biosensors for the optical detection of a pool of sepsis biomarkers, such as procalcitonine and neopterine, with high-sensitivity and low limit of detection, thus breaking a new ground in the biosensors and Lab-on-Chip arena and, in turn, healthcare and point-of-care applications. The choice of sepsis biomarkers as analytes to be monitored with the optofluidic resonant biosensors of this proposal is suggested by the growing request of physicians for Point-of-Care devices capable of performing fast and reliable analysis at patient-level, thus enabling a quick and effective diagnosis and therapy, as opposed to laboratory-level, being the discrimination of viral and bacterial sepsis in intensive care patients or the fast identification of the origin of infections become an essential requirement.

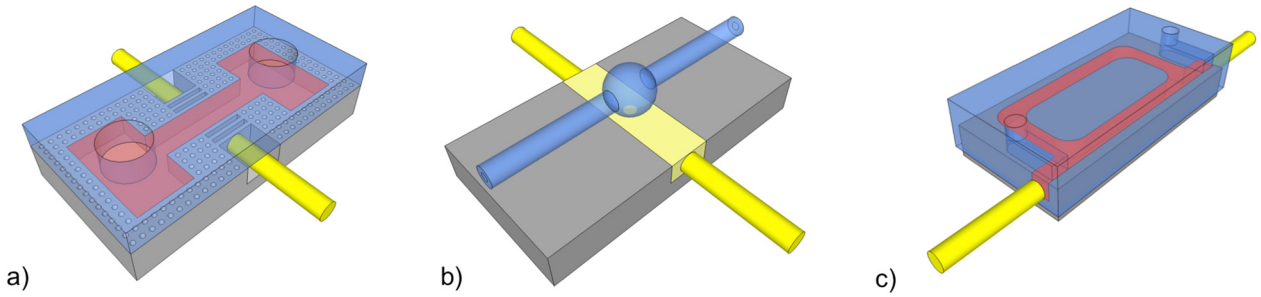

Figure 1. Schematic representation of the three flow-through optofluidic microresonators: a) photonic crystal resonators; b) bubble resonator; c) ring resonator.

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O - 03

MATRIX ASSISTED PULSED LASER EVAPORATION FOR LACCASE BASED BIOSENSORS

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Matrix Assisted Pulsed Laser Evaporation (MAPLE) is a laser-based deposition technique used to deposit films of soft material [1]. In MAPLE, a pulsed laser beam is focused inside a vacuum chamber and impinges on the surface of a rotating target. The target consists of a frozen solution of the material of interest that is diluted in an appropriate solvent. Thus, when the laser beam impacts the target, the laser pulsed energy is mainly absorbed by the solvent and converted to thermal energy, allowing the solvent to vaporize while the material of interest is deposited as a thin film [1]. Therefore, MAPLE is exploitable as an alternative strategy for the immobilization of enzymes [2].

In this study we focused our attention on Laccase, since it is an enzyme widely used as biological recognition component in biosensors for detecting polyphenols that are important compounds in foodstuffs [3] because of their recognized nutritional value.

Laccase has been deposited by MAPLE onto suitable substrates from water and benzene solutions, and the effectiveness of the process has been evaluated in terms of characteristic of chemical bonds (Fourier Transform InfraRed), morphology (Atomic Force Microscopy), enzyme loading (quartz crystal microbalance), and enzyme activity (colorimetric assay).

The deposition process has been also performed onto screen printed carbon electrodes, and the obtained biosensor has been characterized in terms of linearity, LOD, LOQ, repeatability and stability of response. Moreover, the potential of the Laccase biosensor has been also tested by the determination of total polyphenols content in vegetable ethanolic extracts and the results have been compared with those obtained by the Folin-Ciocalteau method.

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O - 04

MICRORING RESONATOR PLATFORM BASED ON FLOW-THROUGH APPROACH

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Microring resonators are emerging as promising building blocks of integrated optics thanks to their versatility in different research fields, such as telecommunication, biosensing and environmental protection and monitoring [1-3]. The small device sizes and the easy of fabrication, with conventional lithographic techniques, make them ideal for large scale integration. A novel fabrication approach include the employment of polymeric materials, for both the substrate and the sensing element, instead of the traditional silicon. The low costs, the high flexibility and ease structuring with micromilling machines makes the polymer materials very attractive for the fabrication of sensing platforms [4-5].
Many efforts were made to optimize the transport kinetic of the analyte under test to the sensing area in order to reduce both detection time and limit of detection. The most interesting method, investigated mainly for suspended nanohole arrays, is based onto "flow through" approach. In that case, the analyte is flowing orthogonally to the sensor surface, so ensuring higher transfer rate respect to standard "flow-over" approach, in which the analyte flows along the surface [6].
In the present work, we developed a novel microring architecture based onto flow-through approach with a fully integrated microfluidic. Su-8 based microring resonators were fabricated on to PMMA substrate with a direct laser writing technique. A drilled hole is realized in the center of the microring resonators by means of a micromilling machine. The biomolecule sensing capability is studied by employing bovine serum albumin (BSA) protein. The sensor response time is evaluated in both in flow-over and flow-through approach, as reported in figure 1. An improvement of about four times of the sensor response time is observed for flow-through approach respect to flow-over, so indicating a better transport kinetic for a real-time detection.

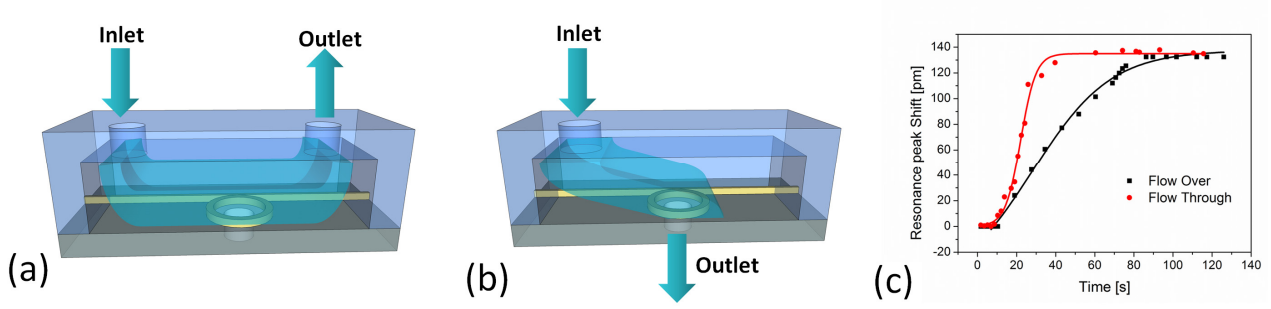

Figure 1. Schematic of the optofluidic ring resonator in (a) flow-over and (b) flow-through configuration; c) response time at a fixed BSA concentration in flow-through and flow-over experiments at 40 μL min^{-1} flow rate.

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O - 05

MOLECULAR BEACON SPECIFIC FOR SURVIVIN mRNA DELIVERED IN CELLS BY PMMA-NANOPARTICLES

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The use of antisense oligonucleotides in gene therapy is a well-known methodology for cancer therapy. An innovative strategy capable to conjugate the ability of sensing specific mRNA with the pharmacological silencing activity, preventing the overexpression of proteins associated to cancer development, may be represented by molecular beacons (MBs), antisense oligonucleotide sequences, which generate a fluorescent signal when they hybridize with their target mRNA. MB is a stem-loop-folded oligodeoxynucleotide with fluorophore and quencher dyes conjugated to the 3' and 5' opposite ends. In the absence of the complementary nucleic acid target, the fluorescence of the fluorophore is absorbed by the closely located quencher. Otherwise, the hybridization with the target opens the hairpin, generating a probe-analyte duplex that physically separates the fluorophore from the quencher resulting in the signal-on after excitation. In this context, nanomedicine is playing an important role offering numerous advantages over conventional drug delivery approaches, improving the imaging, diagnosis and allowing cancer targeted therapy. As delivery vehicles of anticancer drugs, nanoparticles can increase selectivity, reduce toxicity, and prolong the half-life of drugs in the human body with respect to the free drug [1]. In this work, the idea is to use nanoparticles (NPs) as carrier of the molecular beacon, specific for survivin mRNA in A549 cancer cells. Human fibroblasts HDFa have been used as negative control for survivin mRNA expression. The NPs used in this study consist of a core of polymethylmethacrylate (PMMA), with fluoresceine covalently immobilized inside it and the external shell decorated with primary and quaternary ammonium salts. These characteristics make the NPs hydrophilic and biocompatible. The target of the nano-structure under study is survivin mRNA; survivin is a protein belonging to the Inhibitor of Apoptosis Protein family (IAPs) that plays a key role in the regulation of cell cycle, apoptosis and cell migration [2]. Furthermore, its expression is very high in most cancer cells in which the protein levels correlate to poor prognosis and resistance to chemotherapeutic treatment. Even if growing evidence indicates that survivin is also expressed in normal cells, some authors showed that the protein expression is developmentally regulated and very low levels are reported in most terminally differentiated adult tissues [2]. For all these reasons, survivin is considered a promising target for anticancer-drugs.

Acknowledgements
This research study was supported by the national flagship project NANOMAX-ENCODER. S. Tombelli and B. Adinolfi wish to thank the European Community for the projects HemoSpec - Advanced spectroscopic hemogram for personalized care against life-threatening infections using an integrated chip-assisted biophotonic system (FP7-611682) and Nanodem -NANOphotonic Device for Multiple therapeutic drug monitoring (FP7-ICT-2011 - 8318372).

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O - 06

PHOTOLUMINESCENCE ZnO NWs AS A QUANTITATIVE SENSING TOOL FOR MOLECULAR BIORECOGNITION

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ZnO exhibits the richest family of nanostructures (nanoribbons, tetrapods, nanorods and nanowires) among semiconductor oxides and different methods are available to fabricate ZnO nano-objects, including Vapor-Liquid-Solid growth (VLS), Metal Organic Chemical Vapour Deposition (MOCVD), High Pressure Pulsed Laser Deposition (HP-PLD) [1, 2]. However, those methods always require high temperatures, a catalyst and complex equipments that make very expensive and energy-consuming the production of ZnO nanowires (NWs). An alternative approach is the hydrothermal synthesis, an aqueous mediated process to grow ZnO nanostructures, which presents several advantages with respect to the before mentioned: it requires low temperature (60-95 °C), simple equipment and it is possible to grow on a large-area different morphologies of nanostructures, i.e. hydrothermal synthesis is the easier method to obtain ZnO NWs vertically aligned to the surface with a high crystalline quality [3]. Zinc oxide nanowires (ZnO NWs) forest has been grown by versatile hydrothermal method on solid support of very different nature, such as crystalline silicon, glass fiber and polymer surface. ZnO NWs shown a characteristic photoluminescence (PL) spectrum that has been used for the optical transduction of molecular interactions. Under laser irradiation, the ZnO NWs show a characteristic photoluminescence (PL) spectrum composed by a very intense near-band-edge ultraviolet peak at about 380 nm, due to free excitons emission, and broad bands in the visible-near infrared range depending on Zn vacancies, interstitial Zn and lattice defects related to O and Zn, i.e. strongly depending on the preparation conditions.
In this work, we have characterized by optical, label free techniques the interaction between biomodified ZnO NWs, obtained by hydrothermal process, and avidin-HRP. The ZnO nanostructuration was studied by scanning electron microscopy and the biomodification was widely characterized by fluorescence microscopy, Fourier Transform Infrared spectroscopy and wettability change evaluation. Finally, photoluminescence of ZnO NWs was used in monitoring the biomolecular recognition of Avidin-HRP at different concentration and the results show that the photoluminescence intensity is very sensitive to concentration changes. A simple normalization of the emission peaks provided a quantitative monitoring of the biomolecular interaction, revealing an affinity constant in the range of µg/mL per counts and a sensitivity in the range of ng/mL per counts, at least for wavelengths at 380nm and 774nm. These results, as a proof of concept, open a novel route in development of a new useful optical device for label-free bio-medical diagnostic and environmental monitoring.

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O - 07

HIGHLY SENSITIVE LOCALIZED SURFACE PLASMON RESONANCE SENSOR BASED ON MOLECULARLY IMPRINTED POLYMERS AND GOLD NANOSTARS

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Surface plasmon resonance (SPR) and localized surface plasmon resonance (LSPR) is a common tool for surface and nano-surface interaction analysis and bio-sensing, widely used as a detection method for sensors. The methods are able to evaluate small refractive index changes at the interface between a metal and a dielectric medium, which are usually determined by angular or spectral interrogation.

A new optical platform, schematically reported in Fig. 1, is here proposed to achieve this aim by spectral interrogation in a simple and inexpensive way. It has been previously demonstrated to be effective in the case of SPR transduction [1], i.e. using a thin gold layer. Here the same platform is used to exploit the LSPR phenomenon, mainly in order to improve the performance of the sensor in terms of low detection limit.

The dielectric medium in contact with the metal layer is a molecular imprinted polymer (MIP), which at the same time acts as a specific and strong receptor. Upon combination of the analyte with the sites on MIP the refractive index of the polymer changes, producing a variation of the plasmon resonance wavelength, as shown in Fig.1 (transmission spectra). 2,4,6-trinitrotoluene (TNT), an aromatic nitroderivative with low molecular mass, is here considered as a proof of principle.

MIPs present a number of favorable aspects for sensing in comparison to bioreceptors, including a better stability out of the native environment, a better reproducibility and a lower cost. Besides, the refractive index of the MIP here considered (methacrylic acid-divinylbenzene copolymer) is suitable for the proposed platform. The sensing layer contains metal nanoparticles dispersed in the MIP instead of a metal thin film deposited on the optical fiber, thus producing not SPR but LSPR. The shape of the gold nanoparticles is asymmetrical, i.e. a nanostar (GNS) with very thin arms. This should allow a large tridimensional interface between the metal and the polymer [2]. Moreover asymmetrical nanoparticles have several resonance wavelengths, which could improve the sensitivity of the method.

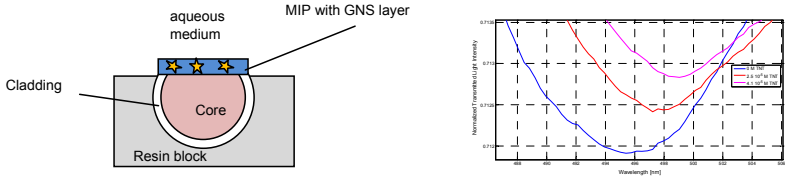

Figure- Schematic cross-section of the sensor, showing the core and the cladding of the optical fiber and transmission spectra at different concentration of TNT.

It is important that the proposed optical platform is based on LSPR in a plastic optical fiber, which presents several advantages over the more classical angular interrogation, and even over the silica fibers.

It has been found that the LSPR wavelength in the proposed configuration depends on the TNT concentration in aqueous solution. The sensitivity and precision is highly improved with respect of the configuration with the gold layer [1].

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O - 08

COMPARATIVE ANALYSIS OF LONG PERIOD GRATINGS-BASED LABEL-FREE BIOSENSORS

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Label-free biosensing based on long period fiber gratings (LPGs) is an emergent sensing technique and could be an efficient alternative with respect to other label-free optical systems, such as surface plasmon resonance, interferometric configurations, and resonating structures[1]. A biochemical interaction occurring on the fiber surface along the grating region will induce a wavelength shift in the LPG transmission spectrum due to the refractive index change of the external environment. In this work, three types of not-coated LPGs, in which the coupling occurs with increasing cladding mode orders, for the increase the RI sensitivity, were manufactured and characterized. A further increase of the sensitivity can be obtained by depositing over the fiber in the LPG region a nm-thick film overlay of RI higher than the cladding RI[2]. For a further characterization and comparison between coated and not coated LPG sensors, two types of sol-gel (titania-silica) coated LPG were also manufactured. The chemical overlay was deposited along the sensing portion of the fiber by means of the dip-coating technique. By changing both the sol viscosity and the withdrawal speed it was possible to adjust the thickness of the film overlay, which is influencing the sensor performances. After the functionalization of the fiber surface using Eudragit L100 copolymer, the same label-free IgG/anti-IgG bioassay was realized and implemented on all the LPGs sensors.

A comparative analysis of the various types of LPG (coated and not-coated) based biosensor was carried out in order to assess and compare the biosensor performance, highlighting the advantages and the disadvantages of each type. The experimental results are summarized in Table 1 (LPG A: sol viscosity: 3.2 mPa s; withdrawal speed 2.5 mm s^{-1}. LPG B: sol viscosity: 27.0 mPa s; withdrawal speed 2.2 mm s^{-1}). The sensors response was also studied using complex matrices made up of human serum.

Experimental results proved an improvement in the RI sensitivity and in the biosensor performance in the case of high-order cladding mode for the not-coated LPGs, while better results were obtained with the sol-gel coated LPGs. A best limit of detection (LOD) of 13 μg L^{-1} (8.6 x 10^{-11} M) was achieved with measurements in serum, which is among the best of optical fiber LPG-based biosensors reported in literature up to now.

	Not coated			*Sol-gel coated*	
	4-th mode	*7-th mode*	*12-th mode*	*A*	*B*
Λ (μm)	615	370	165	*342*	
Bulk Sensitivity (nm RIU^{-1})	1.1	27.9	1298.1	2044.5	7075.3
LOD (μg L^{-1})	7600	500	50	25	13

Table 1. Comparison of the sensing characteristics of the LPG based biosensors.

Acknowledgements

This research study was supported by the Joint Research Proposal (no. 22/EU/Italy/CNR/proj./2012) under CNR, Italy – CSIR, India Bilateral S&T Programme entitled "Development of Long Period Grating (LPG) based immunoassay for bio-sensing applications". S. Tombelli wish to thank the European Community for the projects HemoSpec - Advanced spectroscopic hemogram for personalized care against life-threatening infections using an integrated chip-assisted biophotonic system (FP7- 611682) and Nanodem -NANOphotonic Device for Multiple therapeutic drug monitoring (FP7-ICT-2011 - 8318372). F. Chiavaioli wishes to thank Italian MIUR-FIR program (grant number RBFR122KL1) Microrisonatori Optofluidici Ultra-Sensibili di Tipo "Flow-Through" per Applicazioni Biosensoristiche – SENS4BIO.

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O - 09

A NEW SMARTPHONE-BASED BIOSENSOR FOR QUANTITATIVE CHEMILUMINESCENT-LATERAL FLOW IMMUNOASSAY

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Home self-diagnostic device technology for clinical biomarker monitoring is a growing area of immunodiagnostics. To date, lateral flow immunoassays (LFIAs) integrated with handled sensor devices, i.e. pregnancy test, have been among the most popular point-of-care (POC) applications. However, their diffusion has been limited by difficulties in achieving sensitive and quantitative information using conventional colorimetric or visual color readout. Optical detection based on chemiluminescence (CL) can be an ideal alternative method for POC biosensor development because of its inherent sensitivity and simplicity [1]. Nowadays with the widespread diffusion of smartphones and improved image-processing technology based on back side illuminated CMOS (BSI-CMOS) sensors, fast and accurate POC diagnosis could be developed, based on paper technology CL detection [2,3]. Here, we report on the development of a simple, rapid and accurate biosensor based on a chemiluminescent (CL)-LFIA method applied for quantitative detection of cortisol in saliva, using the smartphone BSI-CMOS photocamera as a light detector [4]. The biosensor is based on a direct competitive immunoassay (Fig. 1a) using peroxidase (HRP)-cortisol conjugate, which is detected by adding the chemiluminescent substrate luminol/enhancer/hydrogen peroxide and by using a smartphone camera for the image acquisition and data handling via a specific application. Using a 3D printer, we made simple accessories to turn a smartphone into a biosensing device. The system comprises a cartridge (Fig. 1b), which houses the LFIA strip, and a smartphone adaptor equipped with a plano-convex lens and a narrow slot for inserting the cartridge (Fig. 1c). When the cartridge is inserted, it creates a minidarkbox making it possible to acquire the CL signals (Fig 1d).

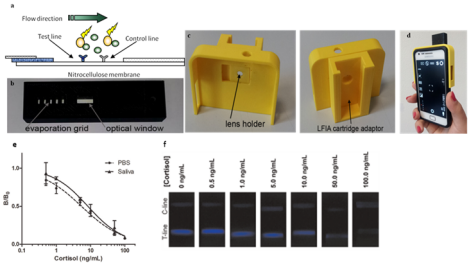

Figure 1. a) nitrocellulose membrane on which reagents for the detection of the analyte were immobilized; b) cartridge that houses the LFIA strip; c) smartphone adaptor; d) integrated cortisol LFIA smartphone-based device; e) calibration curve; f) chemiluminescent images obtained with BSI-CMOS smartphone camera.

The method is simple and fast with a dynamic range from 0.3 to 60 ng/mL (calibration curves are reported in Fig. 1e) that is adequate for detecting salivary cortisol in the normal range and in pathophysiological conditions. The analytical performances of the method were evaluated by comparing results on real saliva samples with those obtained with a validated ELISA kit and a good agreement has been found. In the future, this concept can pave the way for a new generation of portable analytical devices even with a multiplex capability. These kind of biosensors will be useful not only in the medical diagnostic field but in all situations where a decentralized and fast detection is required such as bioterrorism attack, critical medicine, space station, environmental toxicity and analyses in developing countries taking advantages of the peculiar properties of a mobile phone in term of connectivity, location (GPS), long distance transfer of data via wireless.

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O - 10

DRUGS DELIVERY MICRONEEDLES PATCH CONTROLLED BY POROUS SILICON MEMBRANE

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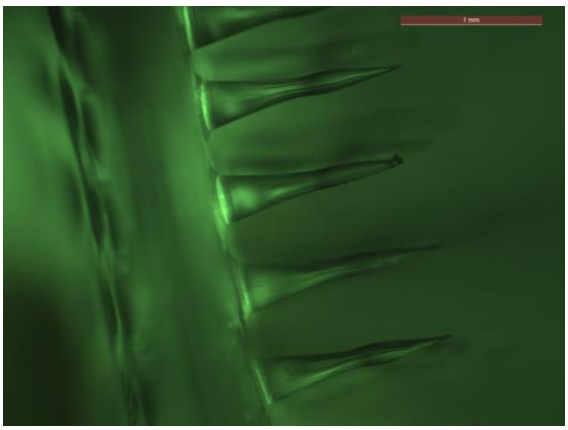
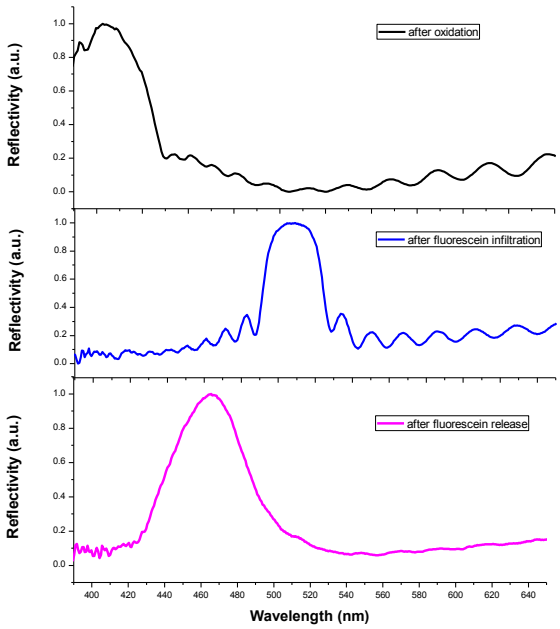
Microneedles are the perfect and painless interface between the derma and a device. Using microneedles as interface and a porous Silicon membrane as reservoir and optical controller, it is possible to realize a smart device for drug delivery. In this work, we present a device based on polymeric MNs. The fabrication of MNs has been realized by using a mixture of PolyEthylene Glycol DiAcrylate (PEGDA) hydrogel and a commercial photocatalyzer. The porous structure of MNs, taken after the polymerization, can include drugs or bioprobes, that diffuse into their polymeric matrix. Also, the device includes a porous silicon membrane with a Bragg's mirror optical structure (PSiBM), whose reflection wavelength is related to the drugs concentration in the MNs.
As proof of concept, in the following the fluorescein molecules has been used. The fabrication of the patch device and the results on the diffusion between PSiBM and MNs and on the release of fluorescein molecules are presenting.
The fluorescence image in fig. 1a clearly shows that the fluorescein molecules are almost uniformly distributed from the base to the top of MNs. A such result proves that the PSiBM effectively acts as reservoir of drugs and the MNs can include molecules. Also, the fluorescein release has been evaluated at hematic pH. The microneedles patch device has been soaked for 2 hours in PBS 1X at pH 7.2. The reflectivity spectra of the PSiBM after oxidation, fluorescein infiltration and release have been acquired and showed in fig. 1b.



a)



b)

Figura 1. a) MNs at fluorescence macroscope. The fluorescein is almost uniformly distributed from the base to the top. b) Reflectivity spectra after oxidation, fluorescein infiltration and release..

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O - 11

BIOSENSING WITH ORGANIC TRANSISTORS FOR DRUG DELIVERY AND IN-VITRO DIAGNOSTICS

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Organic electrochemical transistors (OECTs) are currently emerging as a powerful tool for biosensing, bioelectronics and nanomedical applications owing to their ability to operate under liquid phase conditions, optimally integrating electronic and biological systems. [1] These devices appear to be most suited for biosensing, due to their ability to work at low voltages (< 1V). In this respect, several works dealing with OECTs as sensing devices for different species of analytes, such as hydrogen peroxide, glucose or ions. More complex systems, such DNA, dopamine and cells have been reported in literature, together with some emerging developments in neuroscience such as the control of electronic ion signalling in individual cells, the monitoring of brain electrical activity and the delivering or detecting of neurotransmitters.
The ability to sense and monitor complex bio-structures, such as micelles and liposomes, paves the way for very promising developments in biosensing and nanomedicine. [2,3] Here, we demonstrate that OECTs are effectively very efficient, reliable and sensitive devices for detecting micelle structures and liposome-based nanoparticles. In particular liposome-nanoparticles can be detected on a wide dynamic range down to 10−5 mg/ml (with a lowest detection limit, assessed in real-time monitoring, of 10−7 mg/ml), thus matching the needs of typical drug loading/drug delivery conditions. Furthermore, OECTs are shown to sense and discriminate successive injection of different liposomes, so that they could be good candidates in quality-control assays or in the pharmaceutical industry.
Beyond the capability of detecting a wide class of bioanalytes, OECTs represent also a useful tool for the monitoring of biological-driven phenomena, so that they can be employed as laboratory tool for the study of biomolecules electrochemical properties. We disclose the unique potential of OECTs for detecting and investigating the electrical properties of insoluble eumelanin biopolymers. Gate current measurements on fine aqueous suspensions of a synthetic eumelanin sample from 5,6-dihydroxyindole (DHI) revealed a well detectable hysteretic response similar to that of the pure monomer in solution, with a formal concentration of the polymer as low as 10-6 M. Induction of the gate current would reflect electron transfer from solid eumelanin to the Pt-electrode sustained by redox active catechol/quinone components of the polymer. A gradual decrease in gate current and areas subtended by hysteretic loops was observed over 5 cycles both in the eumelanin- and DHI-based devices, suggesting evolution of the polymer from a far-from-the-equilibrium redox state toward a more stable electronic arrangement promoted by redox exchange with the gate electrode. OECTs are thus proposed as valuable tools for the efficient heterogeneous-phase sensing of eumelanins and to gauge their peculiar electrical and redox behaviour. [4]

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O - 12

LABEL-FREE IMPEDIMETRIC GENOSENSOR BASED ON CONDUCTING POLYMER FOR miRNA DETECTION

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Conducting polymer of biotinylated bis(2,2'-bithien-5-yl)methane is herein investigated for the development of a biosensor for selective electrochemical detection of miRNA sequences. miRNAs are naturally occurring small RNAs (approximately 22 nucleotides in length) that act as regulators of protein translation. Because many diseases are caused by the misregulated activity of proteins, miRNAs have been implicated in a number of diseases including a broad range of cancers, heart diseases, immunological and neurological diseases. Therefore, a great deal of effort has been devoted to develop analytical methods for their detection. In this work, the electroconductive polymer mentioned above was investigated in order to obtain a label-free impedimetric genosensor. The biotinylated bisthiophene monomer was potentiodynamically polymerized to form films on a screen printed gold electrode surface; on the top of the biotinylated films, streptavidin was immobilized by complexing the biotin moieties of the polymer. Afterwards, a biotinylated capture probe was immobilized by complexing the surface-immobilized streptavidin. Total RNA is extracted from the sample and then hybridized with the capture probes. Faradaic impedance spectroscopy (EIS) was chosen as the electroanalytical technique, obtaining a sensitivity in the picomolar range.

RNA samples extracted from non-small-cell lung cancer and breast cancer cell lines were also analysed and results reported.

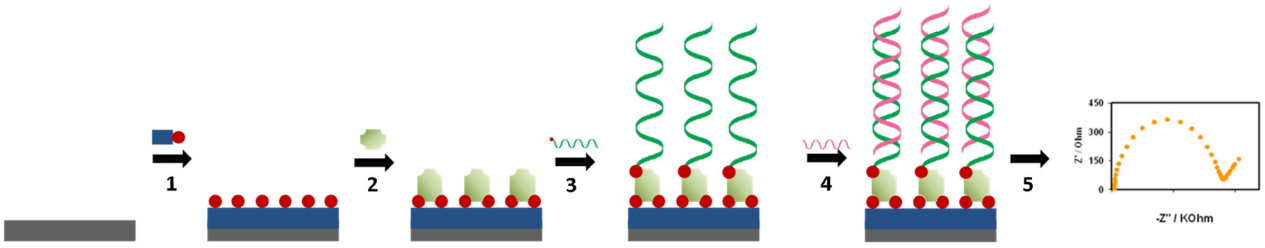

Figure 1. Scheme of the assay. Electrode surface is exposed to a biotinylated monomer water solution and electropolymerization is started (1). The biotinylated film is then exposed to a streptavidin solution (2). Biotinylated DNA capture probe is immobilized on the surface (3). After incubation of target oligonucleotide solution (4) the hybrid is formed on the electrode surface and detected by EIS (5).

O - 13

AN INNOVATIVE IMMUNOSENSOR FOR CO-DETERMINATION OF HUMAN IMMUNODEFICIENCY (HIV) AND HEPATITIS C (HCV) VIRUSES SERUM BIOMARKERS

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Nowadays simultaneous HIV/HCV co-infection occurs with high probability [1], involving heavy consequences for life of people affected. Therefore, it is very important to diagnose the co-presence and to provide an early diagnosis for each of these viruses [2].
In this context, new bio-sensing strategies and platforms were investigated and the research activity was focused on the perspective of simultaneous determination of p24 and SN4 (respectively, HCV- and HIV-related capsid proteins) as diagnostic markers, by using a dual-chip screen printed electrode.
Before using dual devices, p24 and NS4 systems were individually studied to assess their response to the sensor analysis and optimize the setup for each system, then one of these systems, firstly HIV, was studied on mono-chip screen printed electrode.
In the realization of a biosensor an important aspect is the strategy adopted for the immobilization of biological receptors on the electrode surface. In this work a competitive approach was investigated by immobilization of p24 antigen, covalently bound on Carbon Screen Printed Electrodes (CSPEs) by means of chitosan/glutaraldehyde, in order to carry out preliminary studies of the immunoassays.
Two-factors experimental design was performed in order to optimize the concentrations of immobilized antigen and antibody in competition. Data were processed by two-ways ANOVA to study how the tuning of the parameters affects the response and how these variables interact each other. Different concentrations of antigen in competition were explored, obtaining the inhibition curve interpolated by a four parameters logistic function.

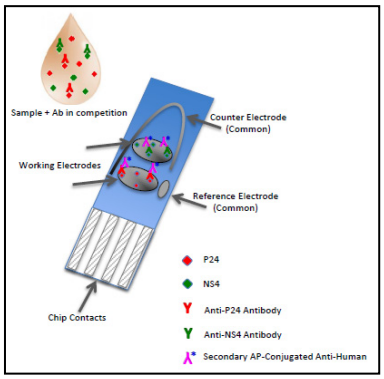

Fig. 1 Dual-chip sensing device

Analogously, chitosan/glutaraldehyde was exploited and optimized for covalent immobilization of NS4 HCV antigen on CSPEs.
Finally, the work was focused on the resolution of a common problem arising from the use of a bi-plexed immunosensor, the so-called "cross-talk" phenomenon [3], taking place due to the diffusion of the electro-active species associated with the enzymatic label used to achieve an amperometric response correlated with the concentration of the analytes. This problem can be overcome by using of a silver salt combined with 3-indoxyl phosphate, leading to a deposited silver that can be electrochemically stripped into solution and measured by anodic stripping voltammetry. This system was also previously studied on a mono-chip screen-printed electrode in order to assess procedure in terms of reagents concentrations and process timing.
After optimization and validation in real samples the studied dual-device could be efficiently applied as rapid method for diagnosis and point-of-care testing.

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O - 14

CONTINUOUS THERAPEUTIC DRUG MONITORING AND SEPSIS BIOMARKERS DETECTION USING THE POCT FORMAT: THE NANODEM AND HEMOSPEC EUROPEAN PROJECTS

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Novel point-of-care-testing (POCT) devices for the continuous measurement of immunosuppressants in transplanted patients and for sepsis biomarkers detection are the final aim of the two European projects NANODEM (NANOphotonic Device for Multiple therapeutic drug monitoring) and HEMOSPEC (Advanced spectroscopic hemogram for personalized care against life-threatening infections using an integrated chip-assisted biophotonic system), respectively.
The new POCT device implemented in NANODEM will allow the automatic measurements of immunosuppressants in transplanted patients leading to the clinical benefit of an optimized dosage of the therapeutic drugs. To this aim, a new TIRF (Total Internal Reflection Fluorescence) based optical biochip for the detection of immunosuppressants was designed. A heterogeneous binding inhibition immunoassay for the detection of the immunosuppressants mycophenolic acid and tacrolimus was implemented. Moreover, a first two-channel prototype chip was manufactured by using two properly shaped polymeric parts (Figure 1).

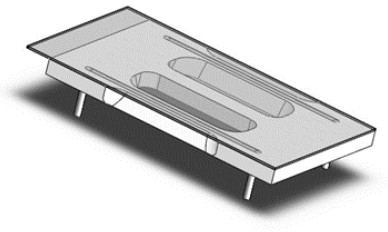

Figure 1. Layout of the two-channel chip prototype for the heterogeneous assay (NANODEM)

The design of a novel POCT biochip for multiple sepsis biomarkers detection is one of the aims of the HEMOSPEC project. High diagnostic potential was found for procalcitonin (PCT), C-reactive protein (CRP), IL-6 among the various interleukins, as well as soluble urokinase plasminogen activator receptor (suPAR), which are the chosen biomarkers in the project. The bioassay implementation for suPAR detection performed on polymethylmetacrylate multichannel chips with a serial fluorescence excitation and interrogation will be presented. A new optical setup is also proposed, which will significantly reduce time of analysis due to the simultaneous fluorescence excitation and read out of all the microfluidic chip channels. This new optical configuration is based on the use of a diffractive optical element to generate a pattern of parallel lines, which will simultaneously excite all the channels. The detection part will be composed of an array of optical absorbing waveguides faced to a large area rectangular detector for the simultaneous filtering of the collected light and detection of fluorescence emerging from each channel.

Acknowledgements
This research study was supported by the European Community within the framework of the projects HemoSpec - Advanced spectroscopic hemogram for personalized care against life-threatening infections using an integrated chip-assisted biophotonic system (FP7- 611682) and Nanodem -NANOphotonic Device for Multiple therapeutic drug monitoring (FP7-ICT-2011 - 8318372).

O - 15

SCREEN-PRINTED ELECTRODES MODIFIED WITH CARBON NANOMATERIALS: A CHALLENGE AMONG CARBON BLACK, CARBON NANOTUBES AND GRAPHENE

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The discovery of fullerene, carbon nanotubes (CNTs) and graphene has impacted very positively in the electrochemical field. The presence of CNTs or graphene on the surface of working electrodes can improve the electron transfer between the surface of modified electrodes obtaining improved analytical performances. Because of advantages of these nanomaterials, various procedures have been developed to produce these nanoscale materials, although sometimes it is not easy to obtain high yields, or concerning graphene, to get one single sheet (pristine) rather than few layers. During recent years another interesting carbonaceous material, but less noble if compared with the previous ones, is becoming interesting for its excellent characteristics and electrocatalytic properties: Carbon Black nanoparticles (CB). CB finds applications as sensitive and filler material, and it is cost-effective (about 1 euro/kg). The advantage to use CB for analyte detection was demonstrated by our research group [1], then several investigations carried out by us and by other groups have confirmed its excellent electrocatalytic properties of CB . In this work a comparative study using Screen-Printed Electrodes (SPEs) modified by drop casting with CB, Single Walled Carbon Nanotubes–COOH, Graphene Oxide, and reduced Graphene Oxide is reported. The carbon nanomaterials employed were characterised by X-ray photoelectron and Raman spectroscopy, while the modified SPEs have been morphologically and electrochemically characterized. Nanoengineered SPEs have been tested with ferricyanide, NADH, ascorbic acid and cysteine in cyclic voltammetry observing a reduced overpotential. Furthermore, the CB-SPE was also tested with NADH, ascorbic acid and cysteine in amperometric mode reaching a detection limit of 1 µM for the analytes tested. We observed valuable electroanalytical performances of CB even when compared to graphene and CNTs, with the advantage to be i) cost-effective ii) suitable to obtain stable and homogenous dispersion and iii) mass-producible following a well established route.

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DEVELOPMENT OF NOVEL ELECTRODE COATINGS BASED ON POLY(2-HYDROXYETHYL-METHACRYLATE)

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The choice of the sensitive element directly interacting with the analyte constitutes the most critical issue in the development of an efficient amperometric sensor. In this respect, it is not excessive to affirm that a notable portion of the progresses in amperometric sensing should be acknowledged to the advent of novel materials for electrodes. The most notable characteristics requested lies in the capability to activate electro-catalytic processes that strongly reduce the overpotential affecting electrochemical processes. This role can be played by a suitable redox-mediator, chosen with respect the specific analyte to detect. Unfortunately, the poor stability of the redox mediator on the electrode surface often represents a drawback for the long-term stability of the sensor system.

In this communication we discuss the performance of a novel electrode coating based on a poly(2-hydroxyethyl-methacrylate) film, stably including different redox mediators through UV photo-induced copolymerization. Thanks to the characteristics of the polymer matrix to swell by incorporation of large amounts of water, redox active species covalently bound to the polymer are in intimate contact with the electrolytic solution. As reported in Figure 1, when ferrocene is linked to the polymer backbone constituting the electrode coating, the progressive incorporation of solution inside the film induces the increase of the ferrocene/ferricinium ion anodic-cathodic current signal. The voltammogram finally shows the typical shape of a diffusion-controlled redox reversible charge transfer.

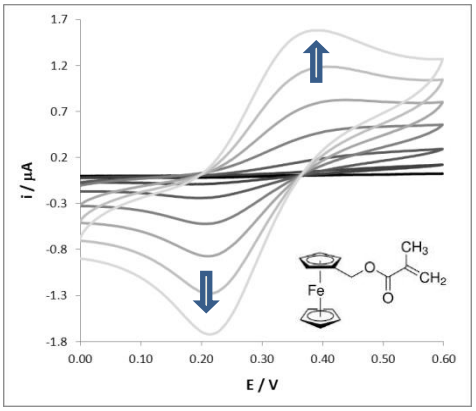

Figure 1. Poly(2-hydroxyethylmethacrylate-co-ferrocenylmethylmethacrylate) modified Pt electrode. Ten subsequent voltammetric scans in 0.1M phosphate buffer solution; 0.02 Vs^{-1} potential scan rate

The material can also form self-standing films that can be mass-produced on an inert support and transferred onto the electrode surface before the use. This process allows the synthesis of a number of electroactive coatings in a few minutes.

The physico-chemical properties of the material, as a function of the chemical formulation and of the synthetic parameters, have been studied by electrochemical, spectroscopic and thermal analyses. The performance of the sensor with respect to the determination of some benchmark analytes has been tested by comparison with the bare Pt electrode and with Pt electrodes modified by poly(2-hydroxyethyl-methacrylate) membranes not containing ferrocene pendant electroactive species.

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ENANTIORECOGNITION TOWARDS L- AND D-DOPA ON EASY-TO-PREPARE INHERENTLY CHIRAL FILM ELECTRODES

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We have recently shown[1,2] that oligomers endowed with "inherent chirality" display high chirality manifestations plus a pool of unprecedented properties. In particular, in the very last months we have demonstrated that electrooligomerization (especially in ionic liquids) of our inherently chiral monomers on screen-printed electrodes and on glassy carbon tip electrodes affords inherently chiral electroactive films of outstanding enantiodiscrimination ability towards a series of chiral probes of quite different bulkiness and chemical nature (also of pharmaceutical interest like DOPA, common antibiotics and FANS)[3]. The general validity of the "inherent chirality" concept has been confirmed by characterizing monomers and related films based on different atropisomeric biheteroaromatic scaffolds (*i.e.* bis-benzothiophene, bis-indole, and "all thiophene" core).

In this work the enantiorecognition ability of our smart films towards L- and D-DOPA will be presented focusing on the variation in voltammetric peak separation of the probe enantiomers when changing *i)* the medium (*e.g.* increasing pH), *ii)* the nature of electrode material and *iii)* the probe carboxylic unit (*i.e.* DOPA methyl ester). The impressive enantiomer peak potential separation combined with the peak current linear dynamic ranges enables to estimate enantiomeric excesses in probe enantiomeric mixtures. Such synthetic electrode surfaces able to neatly discriminate the antipodes of chiral probes as separate peaks are unprecedented in literature, opening the way to the development of efficient chiral voltammetric sensors.

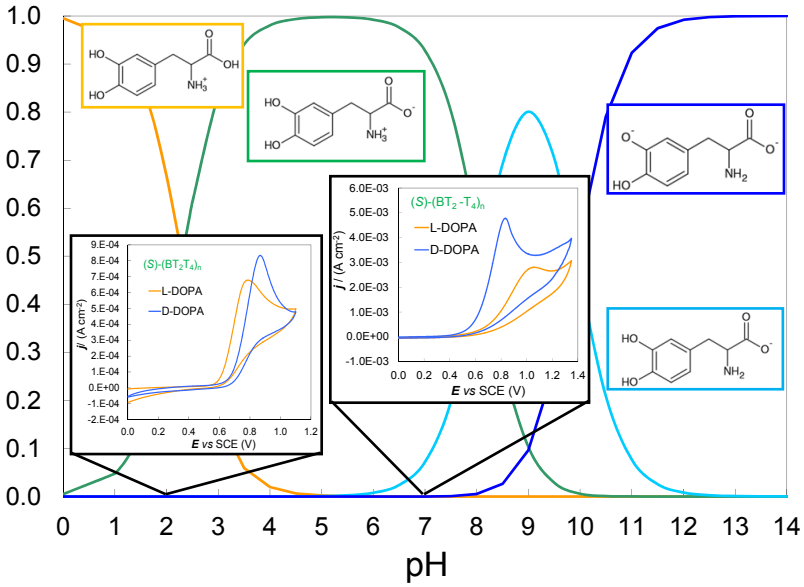

Figure. Effect of the variation in the pH medium on the enantiorecognition ability of our bithianaphthene-based $(BT_2\text{-}T_4)_n$ inherently chiral electrodes with L- and D-DOPA probes, superimposed on a speciation plot of DOPA.

This work was supported by Fondazione Cariplo (Grant no. 2011-0417)

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A NOVEL REAL-TIME, MEDIATOR-FREE, NON-ENZYMATIC ELECTROCHEMICAL BIOSENSOR FOR GLUTAMATE DETECTION

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Glutamate is the major excitatory neurotransmitter in the brain, and an excess of glutamate can cause excitotoxicity, which is a common pathological process in many neurologic disorder such as stroke, brain trauma, and brain tumor. Therefore, monitoring glutamate in real time is of critical importance. The existing biosensors are usually based on the immobilization of glutamate oxidase, an enzyme that converts glutamate and produces hydrogen peroxide; those methods are limited because of the critical step of the covalent immobilization of the enzyme and because they release hydrogen peroxide that is not really suitable for *in vivo* monitoring. In this work, we present a novel mediator-free, non-enzymatic electrochemical biosensor for real-time glutamate monitoring, based on immobilization of genetically engineered periplasmic glutamate binding protein onto gold nanoparticle-modified screen-printed carbon electrodes. Cyclic voltammetry was performed to determine the glutamate concentration in phosphate buffer solution (pH=7.4). The results show an excellent sensitivity with 0.1 µM detection limit and linearity demonstrated in the 0.1 µM-1 µM range of glutamate concentration. The sensor, which was tested with common interfering substances such as aspartate, glutamine, serine, lysine and ascorbic acid, exhibited high selectivity toward glutamate over those substances. Further, the electrode lost only 20% of its sensitivity after a 30 days storage at 4 °C. The comparison between the results obtained with the sensor and those calculated with a commercial ELISA kit will be performed to assess biosensor reliability.
We conclude that a novel, versatile and both enzyme and mediator-free platform, can be constructed with relative ease allowing a sensitive estimation of glutamate.

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O - 19

ALL-PEDOT ORGANIC ELECTROCHEMICAL TRANSISTOR AS A SENSOR FOR ASCORBIC ACID

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Organic Electrochemical Transistors (OECTs) have been proposed as low cost chemical sensors for several analytes thanks to their remarkable features such as signal amplification, the use of an easy and cheap readout electronics and the feasibility on miniaturization[1]. An OECT is composed by a stripe of conductive polymer that works as a channel, and by another electrode, usually a metal, that works as a gate. When the device is dipped in an electrolyte solution, the current flowing in the channel can be modulated through the gate voltage because it promotes electrochemical reactions that change the charge carrier concentration in the polymer and, consequently, its conductivity. Redox compounds can act on such processes by varying the doping degree of the conductive polymer, thereby changing the current density inside the channel.
This contribution describes an ascorbic acid (AA) sensor based on an OECT, made only by poly(ethylenedioxythiophene) poly(styrenesulfonate) (PEDOT:PSS) as conductive material[2]. The development of a sensor for AA determination is important in different fields of science and technology such as medicine, biology and pharmaceutical and food industry. The device (Figure 1) was prepared by spin coating on a glass slide using a commercial suspension of PEDOT:PSS (CLEVIOS^{TM} PH 1000) and the selective deposition on the gate and the channel areas was obtained by delimiting their border with insulating tape. The device was characterized by acquiring AFM images of the PEDOT:PSS surface and recording the characteristic curves. Figure 2 reports the trend of the drain current (I_d) *vs* time while different amounts of AA are added to the electrolyte solution. AA reacts with PEDOT:PSS by extracting charge carriers from transistor channel, and consequently an increase of its concentration leads to a decrease of the absolute value of I_d that results linear dependent on the logarithm of AA concentration between 10^{-6} and 10^{-3} M. The performance of this AA sensor was optimized by varying the gate voltage and the thickness of the PEDOT:PSS layer. The optimized conditions show a sensitivity of 4.5 ± 0.1 10^{-6} A decade^{-1} and a very low limit of detection that is equal to 10^{-8} M. This contribution demonstrates the potentiality of all-PEDOT OECTs as chemical sensors for the determination of redox-active molecules.

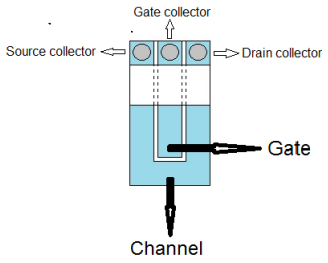

Figure 1. Schematic representation of OECT

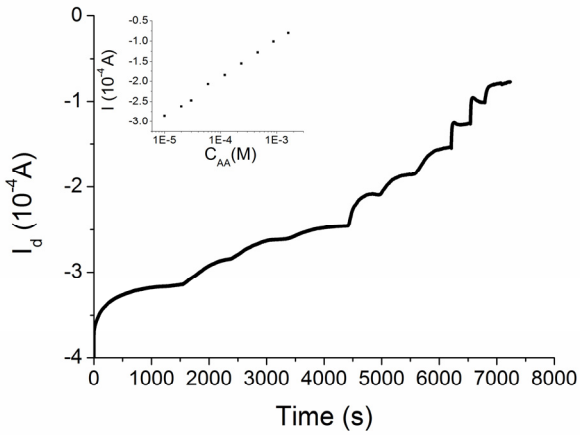

Figure 2. Response of OECT after the addition of different amounts of AA. Inset: plot of I_d *vs* AA concentration (logarithmic scale)

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O - 20

"INHERENTLY CHIRAL" ELECTRODES: TOOLS FOR CHIRAL VOLTAMMETRY AND ENANTIOMERIC EXCESS EVALUATION

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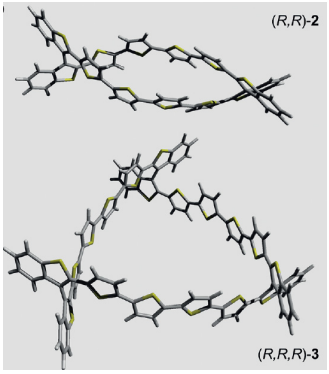
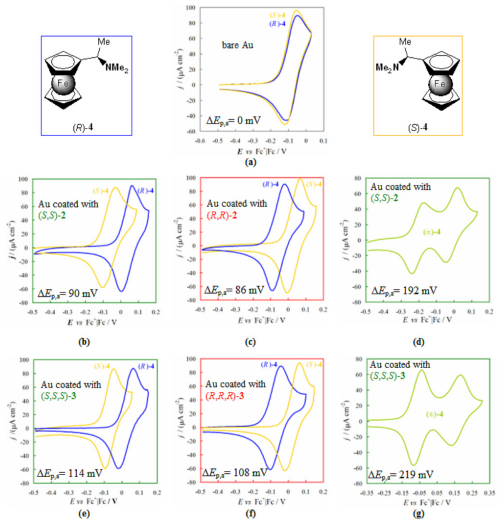
The development of artificial "intelligent" electrodes, capable to discriminate and quantify the enantiomers of chiral analytes, particularly of biological and pharmaceutical interest, is a quite attractive issue in electroanalysis. Obviously, selectivity towards specular molecules can only be achieved on enantiopure chiral electrodes. For this aim, many approaches have been proposed in the last years. However, even the most successful attempts at chiral discimination almost invariably resulted in the detection of a difference in current intensity between the signals of the two antipodes of a chiral probe, without differentiation of their redox potentials; the chiral enantioselective layer is in many instances not of general use, but tailored for a given probe; many preparation procedures are very sophisticated and/or the active films fragile.

A winning solution comes from a new class, which we have recently presented[1-3] and patented[4], of "inherently chiral" molecular semiconductors, whose stereogenic element is a tailored torsion in the electroactive conductive backbone. The coincidence of the element granting both electroactivity and chirality with the entire molecular backbone results in extraordinary chirality manifestations (such as circularly polarized luminescence), that can be finely and reversibly tuned by the electric potential. Above all, enantiopure electrode surfaces can be easily prepared *e.g.* by fast electrooligomerization, mostly consisting of cyclic oligomers, highly electroactive and chiral, idealizing conducting polymers without ends and of high complexing ability; they are able to discriminate enantiomers of chiral molecules in terms of large peak potential differences (80-200 mV and more), with linear dynamic ranges for peak currents, thus affording enantiomeric ratio evaluation. The same spectacular enantioselectivity is obtained on chemically different surfaces of the same structural concept, which demonstrates the general validity of our proposed strategy. A simple reconditioning protocol affords performing more experiments on a single electrode.





The new electrodes have been tested with very good results on chiral probes even very different and of applicative interest[3] (Dopa and methyl-Dopa *[see our parallel presentation]* ofloxacin, norepinephrine, tyrosine, naproxen, catechines, ascorbic acid...), on different supports, including commercial screen printed ones, and in different media (aqueous and nonaqueous ones, as well as small ionic liquid drops on SPEs).

This work was supported by Fondazione Cariplo (Grant no. 2011-0417)

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MESOSCOPIC STRUCTURES AS NEW PLATFORMS FOR SENSING

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The construction of supramolecular structures is determined by a complex interplay of dynamical and structural factors, including the interaction between the molecular building blocks, the differential solvation of monomeric and aggregated species, the presence of chemical groups giving rise to specific site-site interactions (aromatic moieties, charged groups, hydrogen bonding donor-acceptor atoms). All these factors govern the formation of aggregates of different size and morphology (vesicles, fibrils, nanotubes).
In this contribution we report on the formation of 2D and 3D self-assembled nano- and mesoscopic structures of two classes of compounds, i.e. peptide foldamers and steroid-porphyrin derivatives.
Peptide foldamers are oligopeptides assuming few specific constrained conformations. We showed that a single residue substitution dramatically changes the conformational landscape of the peptide building block and this effect propagates to nano- and mesoscopic dimension.[1] Recently, we prepared self-assembled peptide monolayers formed by helical oligopeptides functionalized with a redox active (nitroxide) or a fluorescent (pyeren) group, allowing us to apply, respectively, electrochemical and optical techniques for detection.
In the second example, we will describe how the suitable substitution of a porphyrin scaffold with steroid units can drive the self-assembly process, leading to amplification of physical properties, as the supramolecular chirality and the electric conductivity, to a mesoscopic scale (Figure 1).[2]
The possible application of these systems for sensing will be also discussed at the Conference.

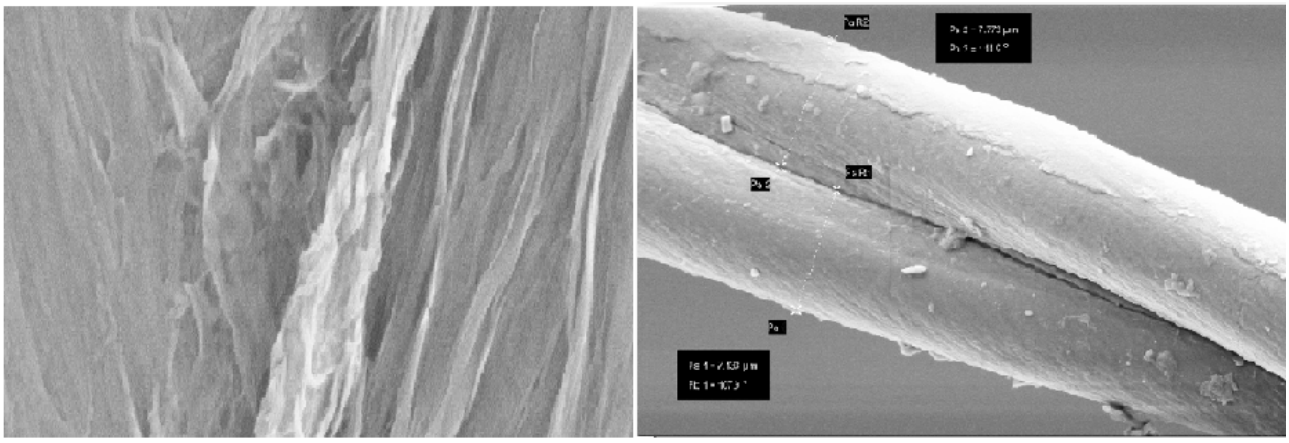

Figure 1. Scanning electron microscopy images of tetrasteroid-porphyrin fibres (left) and columnar rods (right).

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O - 22

HYPERSPECTROGRAMS-GUI: A GRAPHICAL USER INTERFACE FOR FAST EXPLORATION OF DATASETS OF HYPERSPECTRAL IMAGES

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Hyperspectral imaging systems allow to acquire in few seconds tens of thousands of spectra per sample, leading to large-size image files (>50 MB). When a representative sampling of a large number of specimens is required to correctly estimate both intra- and inter-sample variability, this can easily lead to the acquisition of datasets composed by a large number of hyperspectral images and of several GB in size. In this case, the exploration of the dataset by applying Multivariate Image Analysis to single images or to subgroups of merged images does not allow to gain a global overview of the entire dataset variability and to properly highlight the possible presence of outliers, clusters and/or trends. A fast procedure which can be adopted to deal with this issue consists in computing the average spectrum of each image, and then in analyzing the resulting matrix of average spectra. Although this approach can lead to satisfactory results when dealing with homogeneous materials, the information related to spatial variability is lost. By averaging spectra, the useful information related e.g. to the presence of a defect that is localized in a narrow image area could be too diluted within the massive amount of the remainder pixels to be still detectable. With the aim of developing a fast and easy-to-use tool for the exploration of large hyperspectral image datasets, capable of considering both spectral- and spatial-related information of the analyzed images, some of us have recently proposed an approach that allows to automatically convert each hyperspectral image into a one-dimensional signal, named hyperspectrogram [1]. Basically, the hyperspectrogram codifies the information contained in the corresponding hyperspectral image, and is composed by a first part accounting for the pixel distribution (spatial information) and by a second part accounting for the spectral variability. By converting the entire dataset of hyperspectral images into a matrix of hyperspectrograms, it is then possible to compare simultaneously up to hundreds of images by means of common multivariate analysis methods, such as PCA. In order to further facilitate the exploration of datasets of hyperspectral images through hyperspectrograms, we have developed a Matlab Graphical User Interface (Figure 1), which easily allows calculation and visualization of hyperspectrograms, fast exploration of the dataset and visualization of the features of interest contained within each single sample directly in the original image domain.

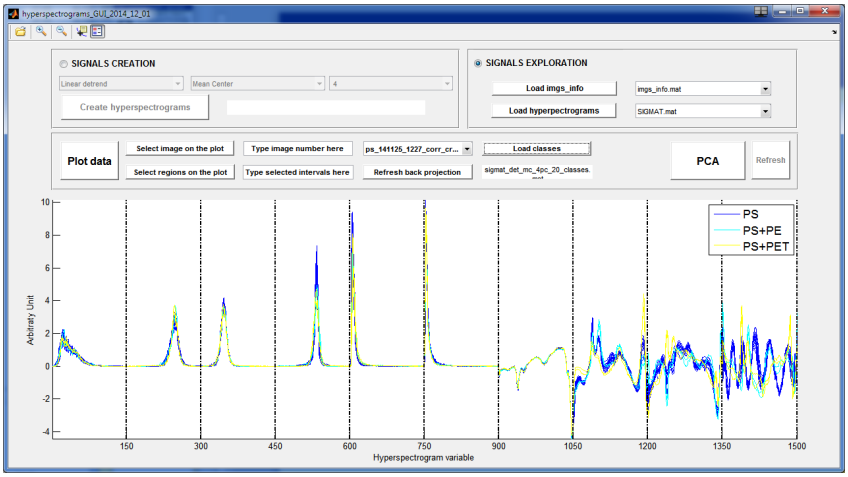

Figure 1. Screenshot of the HYPERSPECTROGRAMS-GUI

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CAVITAND-GRAFTED SILICON MICROCANTILEVERS AS UNIVERSAL PROBE FOR ILLICIT AND DESIGNER DRUGS IN WATER

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Methamphetamines are the fastest growing class of synthetic illicit drugs, which are rapidly replacing heroin and cocaine among drug addicted and occasional consumers alike. Their widespread diffusion constitutes a major challenge for our society, with significant impact on human health and security.
The direct, clean and unbiased transduction of molecular recognition into a readable and reproducible response is the biggest challenge associated to the use of synthetic receptors in sensing. All possible solutions demand the mastering of molecular recognition at the solid-liquid interface as prerequisite.
In this work, we address the socially relevant issue of screening amine-based illicit and designer drugs via nanomechanical recognition at the silicon-water interface. The results demonstrate that the **Tiiii** cavitand targets the $^{+}NH_2$-CH_3 residue present in all methamphetamine salts and, to a lesser extent, the ^{+}NH-CH_3 residue of cocaine hydrochloride with extremely high selectivity in water, thanks to the fine-tuning of CH_3-π interactions and H-bonding. The transduction of the molecular recognition at the interface is achieved with high fidelity, reproducibility and robustness by grafting for the first time the **Tiiii** cavitand on **Si-MC**. The resulting **Tiiii-Si-MC** assay is able to detect the whole class of methamphetamine drugs independently of the type of residue attached to the $^{+}NH_2$-CH_3 moiety, thus opening the way for a sensor capable to single out the entire methamphetamines class. Finally, the **Tiiii-Si-MC** platform is successfully benchmarked by assaying methamphetamines and the corresponding designer drugs against a set of common excipients present in street samples and by detecting the drugs directly in real street samples.

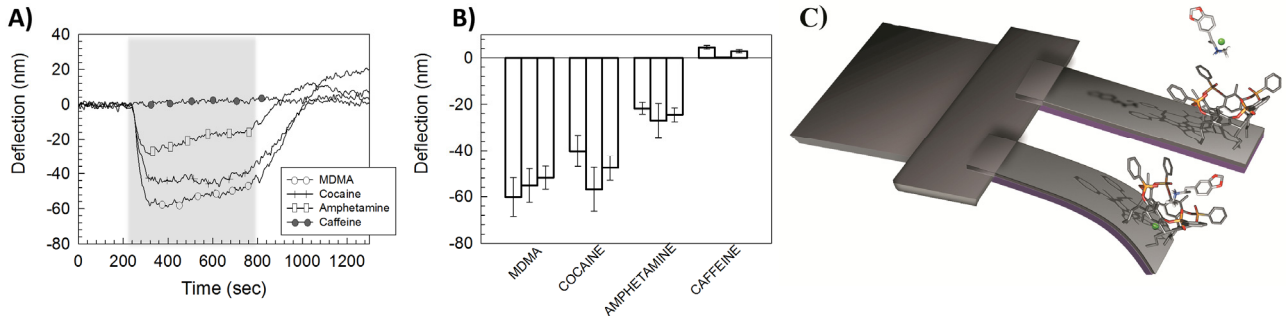

Figure 1. A) Absolute deflection curves of one single **Tiiii-Si-MC** upon injection of illicit drugs. (○) MDMA, (+) cocaine, (□) amphetamine, (●) caffeine. The gray area highlights the injection frame. B) Mean absolute equilibrium deflections of different **Tiiii-Si-MCs** over subsequent replicates. The mean value and the standard deviation (SD) refer to four MCs. Multiple bars for the same sample refer to replicate measurements. C) Representation of **Tiiii-Si-MC**: selective deflection upon complexation of illicit drugs (MDMA in this case).

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DETERMINATION OF ASCORBIC ACID AND PHENOLS RELATIVE CONTRIBUTION TO ANTIOXIDANT ACTIVITY IN FRUIT JUICES BY DIFFERENT SENSOR-BIOSENSOR SYSTEMS

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Fullerenes- or nanotubes-modified graphite sensor-biosensor systems, coupled with a dual channel telemetric device, based on an ascorbate oxidase (AOx) biosensor, were used for on line determination of ascorbic acid (AA), antioxidant activity and to distinguish the relative contribution of AA and phenols to antioxidant capacity in orange, blueberry and kiwi juice. Fullerene C_{60} (FC_{60}), fullerene C_{70} (FC_{70}), single-walled carbon nanotubes (SWCN) and multi-walled carbon nanotubes (MWCN) increased the sensitivity of graphite toward AA and phenols 1.2, 1.5, 5.1 and 5.1 times respectively. Fullerenes combined with AOx improved the selectivity toward AA more than nanotubes, being able to hold a higher number of AOx molecules on the biosensor surface. The systems work at an applied potential of +500 mV, in a concentration range between the LOD and 20 µM, with a response time of two minutes. The LOD is 0.10, 0.13, 0.20 and 0.22 µM for systems modified with FC_{60}, FC_{70}, SWCN and MWCN respectively. Biosensors register lower AA currents than the sensors due to the enzyme capability to oxidize AA before it reaches the transductor surface. Phenols currents registered by sensors and biosensors did not differ. Based on the difference between sensor and biosensor recorded currents a *AA selectivity index* was developed as an indicator of specificity toward AA and of the capacity to distinguish between AA and phenols contribution to the antioxidant capacity. This value is almost zero for fullerene-modified systems, 0.13 and 0.22 for SWCN- and MWCN-modified systems respectively. The results of juices analysis performed with the sensor-biosensor systems were in accordance with reference methods

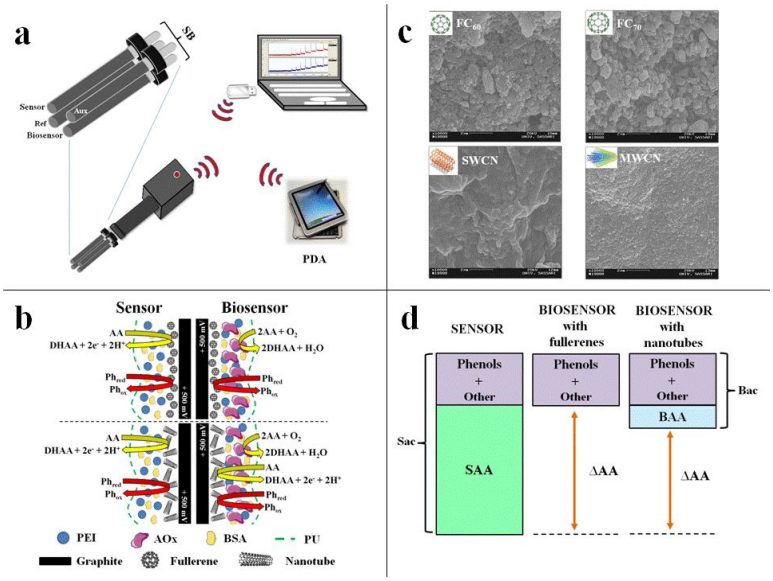

Figure 1. (a) schematic drawing of the telemetric device interconnected with a laptop or with a PDA, (b) SEM images, at 10000 magnification, of the sensor surface nanostructured with FC_{60}, FC_{70}, SWCN and MWCN. (c) schematic drawing of the sensor and the biosensor surface nanostructured with fullerenes or nanotubes. (d) scheme of the working principle of SB nanostructured with fullerenes or nanotubes. Sac = Sensor antioxidant current (is the total current registered by the sensor at the applied potential of +500 mV); Bac = Biosensor antioxidant current (is the total current registered by the biosensor at the applied potential of +500 mV); SAA = is the AA current registered by the sensor; BAA = is the AA current registered by the biosensor; ΔAA = is the quota of AA oxidized by AOx before it reaches the transductor surface of the biosensor.

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CO/AL LAYERED DOUBLE HYDROXIDE COATED ELECTRODE FOR IN FLOW AMPEROMETRIC DETECTION OF SUGARS

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The separation and the detection of carbohydrates is a hard analytical challenge due to the characteristics of these molecules which are highly polar, structurally very similar to each other and do not have chromophoric groups which could favour the revelation step. One of the most employed techniques is HPLC in particular reversed phase chromatography, using bonded phases, ion chromatography, and ion exclusion chromatography. The choice of the detector plays a key role for the analysis of sugars by HPLC: refractive index detectors are the most described, although electrochemical, UV-visible, IR, and evaporative light-scattering detectors have also been employed [1].
The electrochemical detection is based on the analyte oxidation and provides very good sensitivities, higher than those displayed by the refractive index detectors, but a drawback arises from the strong adsorption and accumulation of poisonous intermediates potentially fouling the electrode, partly overcome by pulsed amperometric detection (PAD) [2].
This work reports the development of a Pt modified electrode for the amperometric detection of sugars in flow systems, based on the electrochemical synthesis of a Co/Al layered double hydroxide (LDH). These inorganic materials have a general formula $[M(II)_{1-x}M(III)_x(OH)_2]^{x+}[X_q^{x/q-}\cdot nH_2O]$ and display a 2D structural arrangement. Recently, several applications are attracting increasing interest in the area of electrochemistry, such as batteries, supercapacitors, sensors and fuel cells. Most applications exploit the capability of LDHs containing redox active metals (such as Co and Ni) to undergo an inner redox reaction, within a limited potential range, mostly in alkaline medium. For the Co/Al LDH, it was already demonstrated that Co(IV) centres are able to act as redox mediators for the oxidation of polyhydric substrates [3].
All the applications involving LDHs require the formation of thin films which must be well adherent to the conductive support, and this feature is particularly important when the modified electrode must be employed in flow systems which induce a strong stress to the electrode itself.
A preliminary electrochemical treatment of the Pt surface was carried out in order to improve the adhesion of the LDH film [4] which was confirmed by a bending test which did not display electrode areas in which peeling had taken place.
Some of the most important mono- and di-saccharides have been separated by high performance anion chromatography and detected by the developed device. The best operative conditions which allow the highest detection sensitivity and stability have been established using standard mixtures of glucose, fructose and sucrose. Finally, to assess the applicability of the modified electrode as detector, real samples have been analysed.

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STRATEGIES FOR THE DETERMINATION OF MERCURY TRACES WITH CARBON PASTE-BASED SENSORS

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Carbon paste electrodes (CPEs) are obtained by mixing graphite powder with a hydrophobic binder. The resulting paste can be packed into the cavity of a suitable holder fitted with an electric contact: the resulting assembly can be used as a working electrode for voltammetric and amperometric analysis. The main advantages of CPEs are: i) their versatility: they can be easily functionalized with other materials or reagents (e.g. carbon nanotubes, metal nanoparticles, polymers, ionic liquids, redox mediators, recognition elements…) by simply mixing the modifier with graphite before adding the binder, or by using modified binders; ii) their low cost: they can be home-made with inexpensive reagents; iii) their renewable surface: the cleaning step consists of a simple extrusion of the layer of paste in contact with the sample in order to expose a fresh electrode surface; iv) their wide voltage range, extending from -1.0 to +1.0 V (SCE) [1].

In the present study, we have investigated the suitability of modified CPEs as working electrodes for sensors aimed at the determination of traces of mercury by anodic stripping voltammetry.

In all experiments we used paraffin oil (PO) as binder, 60 mM HCl as supporting electrolyte, differential pulse as potential waveform and 60 s as deposition time. We designed and constructed a Teflon holder from which the layer of paste in contact with the sample can be removed in a reproducible way by turning a screw: after each measurement we discarded such layer and smoothed the new surface with weighing paper. We tested three graphite-binder weight ratios, namely 50:50, 60:40 and 70:30, and found that 60:40 was the most suitable one.

We studied the effect of different modifications on the shape and intensity of the mercury stripping peak:

- use of a ionic liquid (IL), namely 1-butyl-3-methylimidazolium hexafluorophosphate ($BMIMPF_6$), in place of PO or mixed in different ratios with PO, with the aim of exploiting its high conductivity, thus improving electron transfer within the electrode;
- use of two ILs, synthesized at our department on purpose, functionalized with a thiolic group at the end of an alkyl chain (-C6 or -C12) bound to methylimidazolium. The presence of the thiolic group should favour the accumulation of mercury onto the electrode surface and increase the selectivity of the response. In this case, we made experiments with both anodic and cathodic stripping voltammetry;
- electrodeposition of a gold film onto the electrode surface, based on the previous experience of our research group in the functionalization of glassy carbon electrodes with gold nanoparticles[2];
- embedding of gold nanoparticles, previously prepared from $AuClO_4$, within the carbon paste alone or mixed with $BMIMPF_6$. The examination of the electrode material with scanning electron microscopy revealed the presence of sub-micrometric (150-300 nm) gold particles. The most satisfactory results were obtained with this procedure of paste modification, owing to the high affinity of mercury for gold coupled to the easy renewal of the active surface, which led us to prefer the bulk modification of the paste to the electrodeposition of a gold layer.

Work is in progress to optimize the composition of the paste (graphite: PO: IL: gold ratios) and the experimental conditions (deposition time and potential; waveform parameter), with the final aim of developing and validating a sensitive, selective and low cost voltammetric procedure for trace mercury determination with fixed or portable instrumentation, and applying it to the analysis of real samples, namely natural waters and solid matrices (after mineralization) such as food, plants and medicines.

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DETERMINATION OF CAFFEINE @ GOLD NANOPARTICLES MODIFIED GOLD (Au) ELECTRODE

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Caffeine (1,3,7-trimethylxanthine) (CAF) is a natural alkaloid exerting many physiological effects, such as stimulation of the central nervous system, diuresis and gastric acid secretion. It is widely distributed in plant products and beverages and its quantification is mainly of pharmaceutical and alimentary concern.
Many methods, including spectrophotometry, chromatography and biosensing were proposed to this aim. Usually, these methods are generally more expensive, time-consuming and complicated than electroanalytical ones. However, the major drawback of the electrochemical determination of caffeine at the more common electrode materials (e.g., metals, glassy carbon) is that its oxidation occurs at a very positive potential, thus overlapping with the discharge of the background medium, generally not exactly reproducible.
In this paper, we describe a method based on the modification of a gold electrode (Au) surface by deposition of functionalized gold nanoparticles. Preliminary cyclic voltammetric experiments were performed to study the caffeine voltammetric behavior at Au modified electrode in $HClO_4$ 0.4M and in H_2SO_4 0.1 M (Fig. 1A). The oxidation system is characterized by an anodic peak in the positive-going step and by the absence of any cathodic peak on the reverse scan, indicating that the oxidation is irreversible. At the modified electrode, the voltammetric peak height increases vs. that of the bare one, depending on the nanoparticles functionalization. The best performances were observed @ Au electrode modified with colloidal gold nanoparticles (AuNPs) stabilized into a chitosan matrix. We have studied the electrochemical behavior of CAF by means of Electrochemical Impedance Spectroscopy (EIS). EIS measurements are generally used to monitor the interface changes of modified electrodes during the modification process. In the presence of an analyte, the capacitive and resistive features resulting from reactions under study at the electrode/electrolyte interphase are described by the double layer capacitance (Cdl) and interfacial charge transfer resistance (Rct) elements. (Fig. 1B) By using electrochemical techniques, we have explored differences in the CAF redox behavior, which could be reasonably associated both to the different media and to different interactions with electrode materials. Interfering substances such as glucose, ascorbic and citric acid were also studied, under the same experimental conditions.

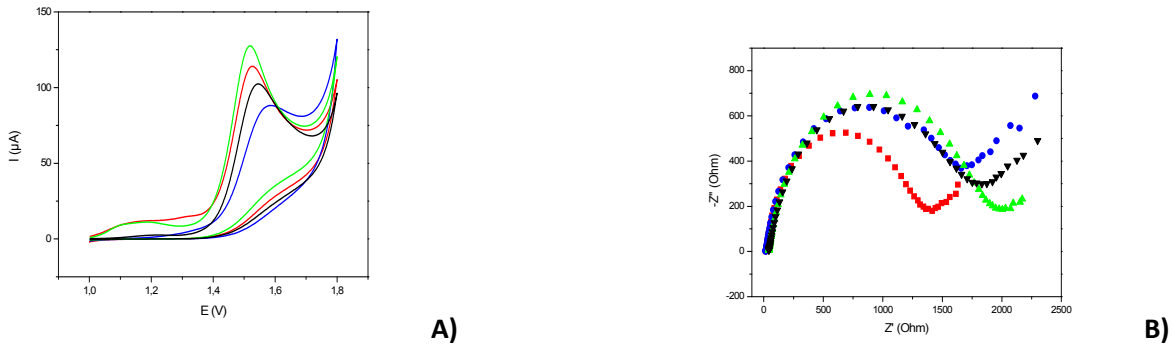

Figure 1. A) Cyclic voltammograms and B) EIS in CAF 5 mM, recorded in $HClO_4$ 0.4M (blue line bare electrode; red line AuNps modified electrode) and in H_2SO_4 0.1M (black line bare electrode and green line AuNps modified electrode)

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NEW FAST AND EASY METHOD FOR THE MONITORING OF Hg CONCENTRATION IN FISH PRODUCTS, USING A GLASSY CARBON ELECTRODE MODIFIED WITH Au NANOPARTICLES

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Mercury is responsible for significant human health problems and environmental pollution, even at low concentration so there is an increasing necessity for the quantification of mercury in different samples, such as in environmental compartments, food, humans and pharmaceuticals. Therefore it is important to develop sensitive analytical methods for its determination. Good results are obtained with electrochemical methods, primarily using anodic stripping voltammetry (ASV), with various working electrodes. Gold is an excellent material for Hg determination since it exhibits a high affinity for Hg, thereby improving the effects of pre-concentration before stripping. Metal nanoparticles can be exploited in electroanalysis for their ability to catalyze the redox processes, since they facilitate the electron transfer; moreover, the large surface area of the deposited nanoparticles could permit an improvement of the analytical performance. This study focuses on the determination of Hg by ASV using a home-made gold nanoparticle-modified glassy carbon electrode (AuNPs-GCE). The performance of this electrode and of the technique have been shown in previous works[1,2] The aim of this work is to continue the evaluation of the possible fields of application of the optimized technique: in this case "fish products".

Modification with gold nanoparticles was performed by dipping the electrode into a 50 mg/l or 100 mg/l of gold (III) chloride trihydrate ($HAuCl_4 \cdot 3H_2O$) solution and applying a potential of -0.80 V for 6 min. In the first step of this study the use of two different $HAuCl_4 \cdot 3H_2O$ salts for the deposition was compared: the salts present different levels of purity declared by the producer: ≥49,0% (used in our previous studies) and ≥99,9%. Repeatability, linearity and detection limit were investigated with the AuNPs-GCE in the presence of different gold nanoparticle layers obtained by changing gold salt and the concentration of gold solution adopted for the deposition. Cyclic voltammetry (CV) has been applied to characterize gold surface with the aims of detecting the presence of gold nanoparticles (strictly related to an anodic peak in the graph) and estimating the amount of deposited gold (related to a cathodic peak) on electrode surface. Moreover CV is useful to observe possible dissolution of deposited gold layer or modifications of its physical structure during the analysis.

The applicability of the AuNPs-GCE for the determination of inorganic mercury in fresh and canned tuna fish by square wave anodic stripping voltammetry (SW-ASV) is demonstrated. Mercury content in sample Tuna Fish ISPRA T22 was determined to value the accuracy of the determination. This sample is a not certified reference material, but literature data are reported for the concentration of mercury in it. The Hg amount was determined also with atomic absorption spectroscopy with graphite furnace (AAS-GF): the results obtained with ASV were in good agreement with AAS results and confirmed literature value. Then real samples of tuna fish were analyzed. The voltammetric analyses were performed using previously optimized conditions (deposition potential 0 V, deposition time XXX s; square wave stripping scan: step potential 0.004 V, frequency 150 Hz, amplitude 0.003 V). The medium exchange technique permitted to eliminate possible matrix effects. The concentrations found in the real samples were in agreement with the common Hg levels reported in literature for commercialized tuna fish in different countries.[3,4]

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O - 29

A SUPRAMOLECULAR APPROACH TO THE DETECTION OF NITROAROMATIC EXPLOSIVES AND NERVINE GAS

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The need of fast, sensitive and accurate real time analyses is the driving force for the development of sensor technology. This is particularly true for homeland security where selective detection at trace level is a must. Currently, the lack of relative specificity of the typical sensing elements makes necessary to use traditional lab analyzers such as GC-MS. Advances in supramolecular chemistry offer many opportunities to design and prepare molecules endowed with superior molecular recognition properties to be used in chemical sensors.[1]

We report a study about the use of quinoxaline cavitand functionalized with a carboxylic group as selective sorbent for analytes containing nitro-groups, providing both pre-concentration and separation capabilities as coating for solid-phase microextraction. The QxCav retains nitroaromatics achieving LOD values in the low ppb_v and ng kg^{-1} range, respectively for air and soil samples.[2]

In a related approach we have designed and prepared new cavitands specifically designed for the detection of DMMP, a simulant for the chemical nerve agent sarin. These receptors, coated on a SPR transducer, are able do detect DMMP at ppb levels and to give extremely low responses to large concentrations of alcohols and water, the major interferents in the environment.[3]

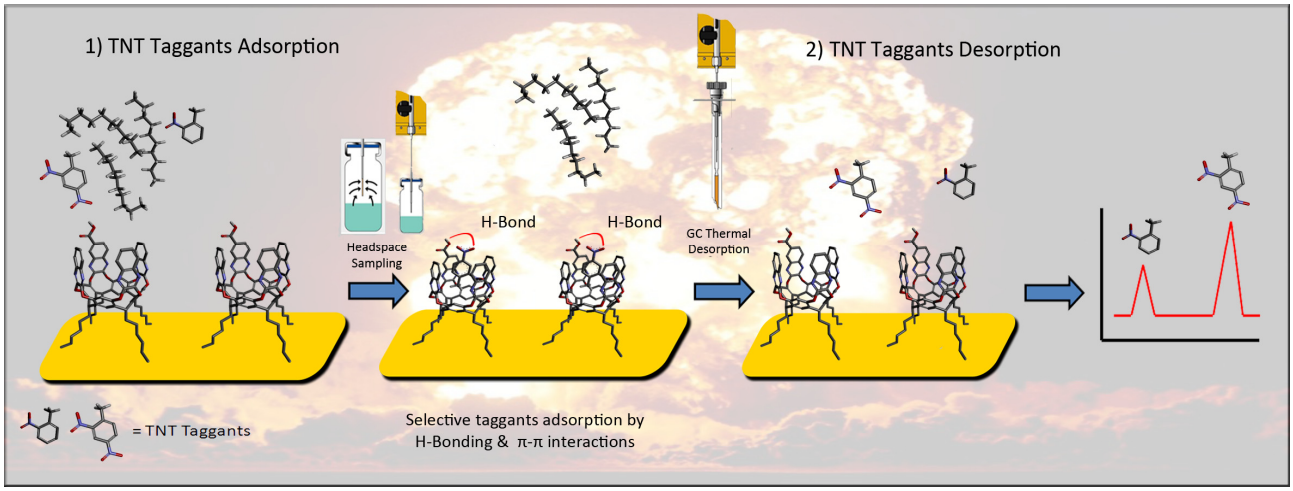

Figure 1. Selective adsorption/desorption process and subsequent GC/MS analysis

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O - 30

ENZYME-FREE JANUS PARTICLES ACCELERATED DEGRADATION AND DETECTION OF ORGANOPHOSPHOROUS NERVE AGENTS USING SPE: A PROOF OF CONCEPT APPROACH

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Bubble-generating microengines have been applied for accelerating chemical reactions and electrochemical detection using printable sensor strips and microliter-sample volumes. Such microengine-assisted electrochemical measurements reflect the greatly enhanced fluid transport associated with the generated microbubbles. The new concept is illustrated towards accelerated degradation with a subsequent electrochemical detection of organophosphorus nerve agents using magnesium Janus microengines coupled with a screen-printed electrode (SPE). The new microengine-based built-in-platform exploits a surprising dual action with solution mixing and control of the reaction parameters, embedded to a sensor strip system capable to fast hydrolysis of the nerve agents towards an easily detectable of the non-hazardous by-product. The disposable platform has been successfully applied toward paraoxon over a wide concentration range (up to 20 mM), allowing its rapid non-enzymatic degradation followed by in-situ electrochemical detection. Such use of Janus microengines can be expanded to diverse strip-based electrochemical sensing applications.

O - 31

ETHANOL AND METHANOL MEASUREMENT USING CATALYTIC DIRECT FUEL CELL: APPLICATION TO ETHANOL DETERMINATION IN WINES AND BEERS SAMPLES

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Our research group was recently developed different biosensors for ethanol (or/and methanol) determination [1,2]. Lastly our team investigated the feasibility of using a direct small catalytic methanol 'fuel cell', obtained from Fuel Cell Store (College Station, TX, USA) and originally constructed for the purpose of obtaining energy from methanol or ethanol, for analytical purposes [3,4].

The aim was to see whether this kind of device can be effectively used for ethanol and methanol determination. In the first experiments, water-alcohol solutions containing increasing percentages of methanol, or ethanol, were added to the cell, the potential increase at the open circuit voltage (OCV), that occurs at two electrodes of the cell was observed and the maximum potential obtained, after each alcohol addition, read off. We thus experimentally demonstrated that it was possible to obtain calibration curves for both methanol and ethanol (see Table 1). The second research step was to repeat the above tests but this time using a potentiostat and recording the current supplied by the cell at different potentials applied to the electrodes. After optimizing, the applied potential operating at a fixed concentration of methanol, or ethanol, measures were made at different concentrations of ethanol or methanol, recording the current supplied, after it had reached a stationary value. The current variation thus obtained was reported as a function of the concentration of the tested alcohol, obtaining suitable calibration curves (see Table 1).

A comparison of the linearity ranges shows that, when operating in OCV format, the linearity range is about two decades while, when operating in potentiostatic format, it is about 1.5 - 2 decades; the LOD is about half a decade lower when operating in OCV format. On the other hand, the measurement time is much lower when operating in potentiostatic format: it is actually at least 4-5 times lower than when operating in OCV format; lastly, sensor lifetime is in any case greater than 2-3 months. The third research step was made carrying out tests, by measuring again the current supplied, although also using enzymes, such as catalase, or alcohol oxidase, or alcohol dehydrogenase, or alcohol dehydrogenase and aldehyde dehydrogenase and NADH as cofactor, inserted inside the anode section of the fuel cell and contained in a small dialysis bag immersed in the ethanol or methanol solution.

Results indicated that the presence of the above-mentioned enzymes actually speeds up the ethanol breakdown process, and therefore enhances the analytical performance of the fuel cell.

In conclusion a fuel cell of the type we tested may be said to be satisfactorily used for analytical purposes and its performance can be improved by using suitable enzymatic catalysts.

Applications to real matrices have been also performed by analyzing the ethanol content of four different commercial samples of wines and three of beers. To validate the obtained results data found using fuel cell, results were compared with those recorded using two other different enzymatic amperometric methods previously developed and pointed out by our group [1].

Table 1. Main Analytical Data

Method	Equations of calibration curves and linearity range (M)		LOD (M)		Response time	
	Methanol	***Ethanol***	***Methanol***	***Ethanol***	***Methanol***	***Ethanol***
Fuel cell OCV format	Y=17.9(±1.8) X + 0.0015(±0.011) R^2= 0.9286 (Y=V ; X=M) $4.5 \times 10^{-4} – 4.5 \times 10^{-2}$	Y=18.8(±1.6) X + 0.005(±0.003) R^2= 0.9491 (Y=V ; X=M) $1.0 \times 10^{-4} – 1.0 \times 10^{-2}$	4.0×10^{-4}	4.0×10^{-4}	5-6 h	5-6 h
Fuel cell RC potentiostatic format polarized at -100 mV VS OCP	Y=21.81(±0.78) X + 0.37(±0.07) R^2= 0.9912 (Y=mA ; X=M) $1.0 \times 10^{-3} – 2.0 \times 10^{-1}$	Y=17.77(±0.95) X + 0.07(±0.02) R^2= 0. 9888 (Y=mA ; X=M) $1.0 \times 10^{-3} – 4.0 \times 10^{-2}$	8.0×10^{-4}	8.0×10^{-4}	~ 60 min	~ 60 min

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O - 32

TEXTILE ORGANIC BIOSENSORS FOR HUMAN PHYSILOGICAL MONITORNG

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Innovative biosensor tailored on the peculiar properties of the materials are gaining interest in research literature. The possibility to grow functional thin films on the specific substrate of technological interest is a crucial key for the commercial application of the functional devices. Organic materials present important characteristics as functional thin film and are useful to improve device on dedicated material. In particular textile fabrics is a wide industrial area with a high request of innovation especially in dedicated wearable functional devices. The idea to grow directly on textile substrates the desired device present the best improvements in the effective working performances which could open important markets and applications. The work presented here actually realize device with active working parts directly grown on textile. In this case we present biosensors based on organic conductive polymer properties. The devices present flexibility, ease of preparation, low cost and biocompatibility [1]. The working principles of the devices are based on a transistor architecture, which allows a high gain in transconductance and opens to different possible configuration measurements [2]. A wide area of possible detection has been demonstrated on devices based on traditional hard substrates: metabolites, ions, neurotransmitters, cells, antibodies and DNA [3].Here we applied organic material on a natural textile surface, realizing a wearable electrochemical transistor. In particular, a single cotton yarn organic electrochemical transistor, low cost, completely integrated e-textile is used as selective biosensor for the detection of human neurotransmitters in human physiological fluids. The process of sensing is analyzed to improve selectivity and sensitivity efficiency. The detection of neurotransmitters in real human physiological fluids could be of crucial importance in the noninvasive analysis of the patients conditions, preventing crisis and attacks. The modulation of the signal response and the kinetic of the signal is used to detect independently biological mixtures. The oxidation process has been studied with UV-Visible absorbance in different conditions. The results confirm that the oxidation reaction is driven by the presence of different metal electrodes. The textile based biosensor demonstrate to monitor human performances, with wearable, non-invasive, low-cost performances, finding large application in sports, health care and working safety.

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O - 33

EVALUATION OF A SYNTHETIC PEPTIDE FOR THE SELECTIVE BINDING OF CHLOROGENIC ACID DERIVATIVES USING ELECTROCHEMICAL SENSORS

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The aim of this work was the evaluation of the binding capacity of a synthetic peptide, designed by a computational approach, towards a series of phenolic compounds involved in food chemistry. The peptide was designed allowing two possible anchoring site for chemical binding to sensor surface: a glycine residue that can be coupled to carboxylic groups and a cysteine for direct immobilization on metals surface such as gold electrodes. The peptide affinity towards caffeic acid, used as model target molecule, was performed following the fluorimetric quenching of a tryptophan of the peptide sequence upon binding with the target molecule. In order to apply this peptide to the development of an electrochemical sensors for the selective determination of phenolic compounds in food, we carried out a screening of possible interfering analytes using cyclic voltammetry and differential pulse voltammetry as detection strategy. To avoid electrode fouling disposable screen printed electrodes were used for electrochemical detection. The study was carried out in buffered solution at pH 7.0 without the addition of organic solvents. In the presentation results on the affinity of the peptide towards different phenolic structure will be shown.

POSTER

P - 01

ELECTROCHEMICAL BIOSENSOR FOR POLYBROMINATED DIPHENIL ETHERS (PBDEs) DETECTION.

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Polybrominated diphenyl ethers (PBDEs) are persistent environmental substances that have been commonly used as fire retardants in huge number of commercial products. Their ubiquity in the air, water, food due to their low reactivity, high hidrophobicity and bioaccumulative properties causes a continuous exposure to these compounds. Moreover PBDEs are known to cause severe health problems and thus the commercially available mixtures have been banned from the market.
The objectives of this study were to provide updated measurements of PBDEs in food by GC-MS analysis, to estimate possible difference in levels from differing types of food samples and to afford an improved estimate of current dietary intake. Moreover, the suitability of using a magnetic particle enzyme-linked immunoassay (ELISA) to analyze PBDEs in food samples was also tested. An electrochemical multiplexed biosensor for the simultaneous detection of PBDEs and PCBs (polychlorinated biphenyls) was also developed. Food samples were randomly acquired in breeding farm and slaughterhouse. Samples were ASE extracted, cleaned up on a Power-Prep system and finally analyzed.

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P - 02

SMARTPHONE EMBEDDED ENZYMATIC COLORIMETRIC/CHEMICAL LUMINESCENCE-BASED BIOSENSOR FOR POINT-OF-NEED APPLICATION

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We present the use of a non invasive biosensor based on smartphone to image and quantify biospecific enzymatic reactions coupled with bio-chemiluminescence [1, 2] to detect analytes in saliva and sweat. To this end we implemented a simple and compact portable disposable minicartridge into a smartphone placed in front to the photocamera used as a detector. The production of photons by a chemical reaction facilitates the biosensor configuration which simply consists of a nitrocellulose disk in which the analyte biospecific enzyme, usually oxidases, is co-immobilized with the light producing enzymes peroxidase using luminol/enhancer as a luminogenic substrate. As proof-of-principle [3], lactate oxidase was coupled with horseradish peroxidase for lactate determination in oral fluid and sweat. The minicardridge was fabricated with use of a low-cost commercial 3D printer and consists of three separate components: (a) a disposable analytical cartridge, (b) a mini dark box, and (c) a smartphone adapter that is equipped with a plano-convex lens that allows to focus the spot image. The analytical cartridge contains two reaction chambers (sample and control reaction chamber) with small disks of the nitrocellulose membrane, onto which LOx and HRP are co-absorbed. Lactate can be quantified in less than five minutes with detection limits of 0.5 mmol L^{-1} (corresponding to 4.5 mg dL^{-1}) and 0.1 mmol L^{-1} (corresponding to 0.9 mg dL^{-1}) in oral fluid and sweat, respectively. Alternatively, the chemiluminescence–based detection of lactate quantification can be obtained using a reagentless bioactive paper-based solid-phase biosensor integrated in smartphone-based device exploiting colorimetric detection. The device differs from the above mentioned one by the presence of a unique reaction chamber in the analytical cartridge, smartphone flash as light source and a PDMS-based light diffuser. The latter allows to obtain a more uniform brightness of light for detection area. The assay support is composed of a cellulose paper onto which enzymes (HRP and Lox) and a chromogenic substrate (TMB) are entrapped in a bilayer component polymer coating of poly(styrene sulfonate) (PSS) and poly(allylamine hydrochloride) (PAH). The chemical modification of cellulose allows to improve the homogeneity of the color distribution in the detection zones, avoiding the diffusion of reagents. Exploiting the light reflectance principle, color analysis based on HSV colorimetric system was performed using a dedicated smartphone application allowing to correlate the signal to the analyte concentration. This biosensor configuration provided slightly higher limits of detection in comparison with those obtained with the CL biosensor but with reduced analysis time (1 min vs 5 min) and cost (no need for reagents).

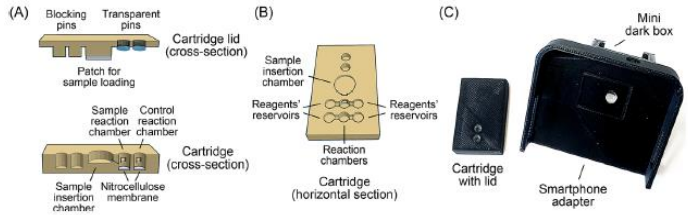

Fig 1. (A) Cross sectional and (B) Orizontal view of the 3D printed cartridge for CL detection (cartridge for colorimetric detection has only one reaction chamber); (C) Photo of the cartridge-lid assembly and smartphone accessory.

Therefore both the developed smartphone-based biosensors could find application for cost-effective non-invasive lactate measurement to monitor the intensity and the maximum duration of athletes' performance during physical exercise. The devices, combining mobile phone connectivity, GPL, Wifi technology with facile 3D printing, could be easily adapted to a variety of other assays utilize other oxidases enzymes that require simplicity, low-cost, portability, and flexibility.

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P - 03

PDIF-CN_2 MODIFIED POROUS SILICON OPTICAL AND ELECTRICAL TRANSDUCER FOR BIOCHEMICAL SENSING

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In this work we report our preliminary results on porous silicon (PSi) non-symmetric microcavities as optical transducers modified by Joule evaporation of a conductive material, called PDIF-CN_2. Even if PSi optical transducers are used by more than twenty years in biochemical sensing, their features are still attractive for a great number of academic and industrial researchers [1]. Since PSi transducing mechanism is based on air substitution by liquid or solid matter infiltrated from external, chemical or biological functionalization procedures are mandatory in order to obtain a specific and selective optical sensor. Organic molecular beam deposition of PDIF-CN_2 improves electrical conductivity of PSi transducer, making it a double signal sensor. In the field of organic semiconductors, PDIF-CN_2 is well known for its interesting charge transport properties, making it particularly attracting for the development of high-performance organic filed-effect transistors (OFET) and related circuits [2]. PSi photonic structures were fabricated by electrochemical etching of p+ crystalline silicon in hydrofluoric acid (HF), water and ethanol solution, in dark and at room temperature. Non-symmetric microcavities are constituted by a particular sequence of PSi layers: HLHLHLHLHLHL LL LHLHLHLHLHLHLHLHLHLHLHLH, where H and L are high and low refractive indexes. One microcavity is thermally oxidized in order to protect it against uncontrolled environmental aging and corrosion in alkaline solutions; the other has been used as freshly etched. PDIF-CN_2 has been vacuum deposited on the two devices at room temperature, by creating a layer with thickness of about 30 nm. PSi infiltration by PDIF-CN_2 has been monitored by reflectometric spectroscopy: after PDIF-CN_2 deposition, there is a red-shift of optical reflectivity spectrum of about 6 nm for the fresh etched microcavity and 5 nm for oxidized microcavity (see Figure 1). We have then performed time-resolved measurement in order to characterize devices dynamic: a laser beam with 785 nm wavelength impact on device, than reflected light goes to the photo-detector that sends the signal to an oscilloscope [3]. The signal was measured, as a function time, before, during and after the exposure to the gaseous substances. On exposure to iso-propanol vapours, due to the phenomenon of capillary condensation, the average refractive index, and therefore the optical thickness, of the layer increases. Rise and fall times measured are about 14.3 s and 3.6 s for fresh etched microcavity, 4.4 s and 21.3 s for oxidized microcavity. The next step of this work is to study the electrical properties of PDIF-CN_2 modified PSi-based microcavities, in order to show that organic semiconductor improves them.

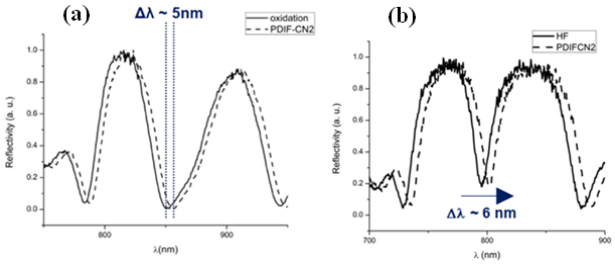

Figure 1. (a) PSi optical reflectivity spectra after oxidation and after PDIF-CN_2 deposition; **(b)** PSi optical reflectivity spectra after refresh in HF and after PDIF-CN_2 deposition.

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P - 04

POLYMERIC MICRONEEDLES INTEGRATING GLUCOSE AND LACTATE SENSOR

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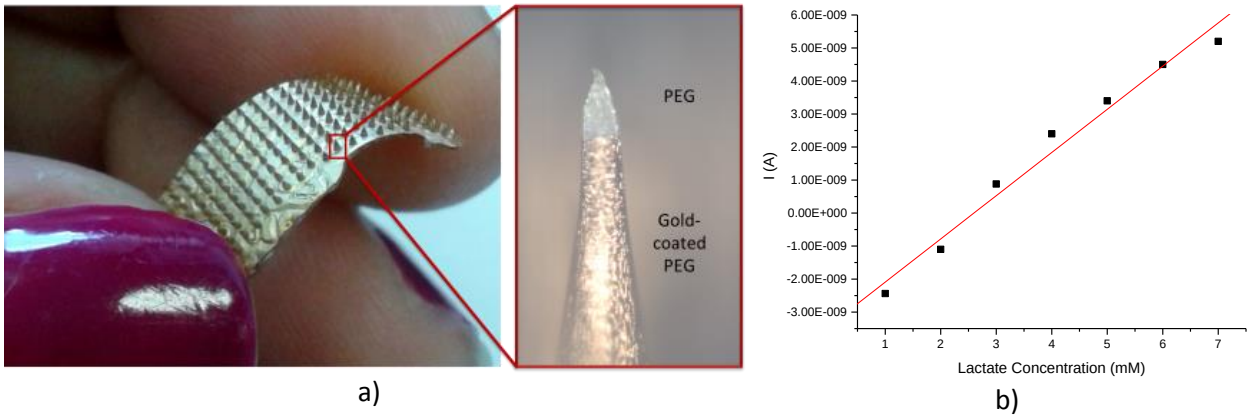
Applications of microneedles in biomedicine wide range from diagnostics to therapeutics. This crucial and versatile tool is the interface between the human body and often a complicated device. We present a sensing device based on hybrid microneedles array for diagnostic applications. The hybrid microneedles are made of a photo-definable polymeric hydrogel. They were fabricated by using a commercial photoinitiator to cross-link a liquid PolyEthylene GlycolDiacrylate (PEG-DA). After polymerization, the MNs have a porous structure, which can include a variety of biological molecules, as bioprobes or drugs. The presented device is an electro-chemical sensor, where microneedles include proper enzymes in their matrix that interact with lactate or glucose. The working electrode is fabricated by plating with gold the MNs and etching their tips (fig.1a). The redox reaction with the analyte, mediated by ferrocene, creates a charge transfer resulting in a current proportional to the analyte concentration (fig.1b).



a) b)

Figure 1. a) Gold-coated MNs array in PEG. The insert shows the uncoated tip reacting with the glucose solution b) Preliminary results: electro-chemical current versus lactose concentration.

ACKNOWLEDGMENT
This study has been financially supported from MIUR Ministry (Italy) within the FUZI project (PON02_00029_3148467).

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COMMON SENSE PROJECT – "COST-EFFECTIVE SENSORS, INTEROPERABLE WITH INTERNATIONAL EXISTING OCEAN OBSERVING SYSTEMS, TO MEET EU POLICIES REQUIREMENTS"

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The objective of the current work is the introduction of the European COMMON SENSE project (http://www.commonsenseproject.eu/), aimed to support the implementation of European Union marine policies such as the Marine Strategy Framework Directive (MSFD) and the Common Fisheries Policy (CFP). The project has been designed to develop and provide cost-effective and multi-functional innovative sensors to perform reliable *in-situ* measurements in the marine environment. The core project research will focus on increasing the availability of standardised data on: eutrophication, concentration of heavy metals, microplastics fraction within marine litter and underwater noise. Furthermore, the project will also address additional new sensors for innovative pyro and piezo resistive polymeric temperature and pressure sensors and nanosensors for autonomous pH and pCO_2 measurements.
The specific objective of CNR-IPCB is the development of devices for pH and pCO_2 detection in marine environment. In particular, the design and development of microcomposite and nanocomposite films will be carried out aiming at the realization of cost-effective pH-sensor for acidification research of seawaters as well as for environmental monitoring.
Multi nanosensors will be developed with a new combination of chemical sensory and structural functions and they will be able to detect pH and low levels of CO_2 gas, as well as to monitor structural changes. Successful development of these new devices would overcome size and cost problems associated with current nanosensors used in research, and open the way to their mass market. Special fillers such as multi-walled carbon nanotubes and graphene will be used since their special properties offer great potential applications as chemical and mechanical sensor devices with excellent sensitivities and fast responses.
The detection of the parameters mentioned above is important since pH and pCO_2 can be correlated with eutrophication: in particular, a decrease in pH value can be due to an increase in the emission of CO_2 in eutrophic waters, enhancing so the effects of ocean acidification by atmospheric CO_2 uptake.
Further Institutes, CNR-ISMAR and CNR-IAMC, will participate to the project by providing expertise in needs and requirements for sensors to be interoperable with existing observing systems, in platform availability, adequacy and difficulties. They will be responsible for the design, coordination and implementation of the field-testing activities and for the platforms sensor deployment.

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P - 06

DEVELOPMENT OF A NANOSTRUCTURED PRESS TRANSFERRED ELECTRODE COUPLED TO MICROFLUIDIC ELECTROPHORESIS, FOR FOOD CONTAMINANTS DETERMINATION

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The aim of this work is the development of a rapid and quantitative multi-residual screening method , for the separation and detection of carbamate pesticides in food samples. To this aim a simple method for the fabrication of electrodes based on carbon black (CB) is presented. For the first time the press-transfer (PT) technique[1,2] was used with the nanostructured CB, that act as exclusive electrochemical transducers. The production of a stable CB nano-dispersion does not require sophisticated procedures; due to this reason combined with the low cost of the material, in recent years the use of CB as a nanomaterial has found an increasing application in electrochemical sensing [3-5]. CB dispersion was filtered through a PTFE membrane and press-transferred on polymethyl methacrylate (PMMA) substrates. To make possible the separation of the analytes, the electrodes have been designed to be coupled to microchip electrophoresis. The optical and electrochemical characterization was performed both off-chip and on-chip.

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P - 07

DEVELOPMENT OF A BIOSENSORISTICS TELEMETRIC DEVICE APPLIED TO MICROBREWERIES IN SARDINIA

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The beer is currently the most consumed alcoholic beverage in the world and the global beer production has had a rapid growth and gained great success also in the countries that are not traditional beer producers (1); more than 900 craft breweries are known just in Italy. Growing evidence supports the health benefits of moderate consumption of alcohol as part of a healthy lifestyle, whereas it is widely recognized that alcohol abuse exerts negative effects on health. A plethora of studies suggest that moderate drinking of beer is associated with lower rates of cardiovascular disease, neurodegeneration, cancer and osteoporosis (2). The positive association between moderate intake of beer and low risk for these diseases is due to several substances with antioxidant properties present in the barley and in the hop (3). Also, hop contains some volatile oils which show antibiotic activity (4). The nutraceutical property of the beer is preserved if it is manufactured by a minimal invasive procedure like the brewing craft is. In order to stabilize the composition, the industrial procedures use high temperatures and filtrations techniques but resulting in a poor nutritional and health properties. However, the wholemeal composition of handcraft makes the product more susceptible to contamination throughout the brewing process, putting at risk the success of the final product (1).
Analytical techniques such as HPLC, GC, GC-MS are widely used for the monitoring the main analytes that take places under the manufacturing process conditions. However, they are time-consuming and laborious and, in some of them, the sample pre-treatment is required (5).
Methods based on biosensors could be a good alternative to these classical methods. They offer fast analysis, easy and direct analytes detection and low cost production. Amperometric biosensors seem to be the most commercially successful devices to this purpose.
Amperometric biosensors applied to beer production have been investigated in our research labs. They are able to detect the main fermentation compounds like glucose and ethanol with high sensitivity and repeatability. Microbial contaminants such as lactic acid can also be detect below the thresholds of perception (100-400ppm). Furthermore, a remote control has been designed. The wireless technology allows to send in real time the signals obtained by the analysis performed *in situ* (6) without having to send the sample to the outside of the craft brewery.
Thus, the microbrewery can check and modify the process conditions in an easier, faster and less expensive way.

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P - 08

FUNCTIONALIZED GOLD NANOPARTICLES EMBEDDED IN POLYMERIC MATRICES FOR SENSOR DEVELOPMENT

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Nanoscience and nanotechnology are growing fields, which are gaining great impact in different areas, ranging from electronics and communication technologies to biomedical engineering. The production and the use of nanomaterials, such as nanoparticles (NPs), is playing a central role in recent years, due to their unique optical, electrical, and magnetic properties that are often not observed in the bulk materials.[1] NPs can act as signal enhancers or can be used to immobilize, *via* a covalent linkage, an enzyme/protein and/or a drug, in order to obtain stable bio-conjugate systems, for exploring their potentials in the development of biosensors. Among nanoparticles, gold nanoparticles (AuNPs) are the most stable metal nanoparticles, and have been widely employed to design biochips and biosensors used as analytical tools.[2-4]

AuNPs can be embedded in many different polymer matrices, and these nanocomposites may exibit enhanced properties, derived from the combination of both nanoparticles and polymer properties, and strictly related to the shape and size of the AuNPs.

In this contest, we propose nanocomposite polymeric materials, based on functionalized AuNPs, for the development of a biosensor for glucose detection.

In particular, the proposed materials are made up of polyethylene glycol (PEG) containing AuNPs conjugated with Glucose Oxidase, GOx, and artificial metalloenzymes with peroxidase-like activity.[5]

The synthesis and the spectroscopic, functional and morphological characterization of the new nanobiomaterials will be presented.

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P - 09

SMARTPHONE-BASED COLORIMETRIC ASSAY FOR CA125 CANCER BIOMARKER DETECTION

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There is an urgent need for cost-effective point-of-care (POC) instruments with homogenous technical requirements as well as more flexible devices for biomarker diagnostics in clinical settings. Due to the recent advances in smartphone features (such as capability, processing power, hardware and software), it becomes a promising tool for mobile diagnostic and bio-analytical POC tools. Colorimetric detection is an ideal method for miniaturization and POC biosensor development because of its inherent sensitivity and simplicity.

In this work, we have developed a simple and accurate affisensor based on a colorimetric immunoassay method coupled to a smartphone in order to detect quantitatively the ovarian cancer antigen 125 (CA125). The affisensor is based on a sandwich immunoassay in which the primary antibody was immobilized by spotting the antibody solution on nitrocellulose membrane. Subsequently, the spots were incubated with CA125 antigen followed by affinity reaction with a secondary antibody conjugated to gold nanoparticles (AuNPs). The silver enhancement reaction was introduced to magnify the signal detection. The experimental data show that this reaction can be observed by the naked eye. The formation of gold-silver nanoparticles results in a different grey colour, depending on CA125 concentration. The smartphone camera was used as colour detector, for image acquisition and data handling via a specific application.

The parameters involved in each step of the affisensor design were optimized. The performance of the immunoassay in terms of sensitivity, reproducibility and selectivity was studied.

Under optimal conditions, a linear response was obtained in the range of 60 – 1000 U/mL, which is also the important range from clinical point of view. The method is simple, fast, and can be performed without requiring highly skilled operating personnel and expensive instrumentation allowing point-of-care analysis with reductions in cost and response time.

P - 10

NANOSTRUCTURED DNA APTASENSOR FOR ACETAMIPRID ANALYSIS

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In this work, an electrochemical DNA-based array for acetamiprid, a diffused neonicotinoid insecticide, is presented. The DNA aptasensor is based on a dual signal amplified strategy by employing a polyaniline film and gold nanoparticles as sensor platform and an enzyme-linked label for sensitive detection. Firstly, polyaniline film and gold nanoparticles were progressively grown on a graphite screen-printed electrode surface via electro-polymerization and electrochemical deposition, respectively.
The polyaniline-gold modified surfaces were then modified with a mixed monolayer of a thiol-tethered DNA aptamer and a spacer thiol. The aptasensor was able to capture the pesticide from the sample solutions. An enzyme-amplified detection scheme, based on the coupling of a streptavidin-alkaline phosphatase conjugate and biotinylated secondary aptamer was then applied. The electro-active enzymatic product was detected by means of differential pulse voltammetry. The sensor coupled the strong advantages of the enzymatic amplification with the electrochemical properties of polyaniline and gold nanoparticles. Various experimental parameters of the realized DNA-based nanostructured sensor were studied and optimized using optical and electrochemical techniques. A calibration curve between 0-1000 nM acetamiprid concentration range was obtained.

DETECTION OF ORGANIC POLLUTANTS IN WATER: WHAT MATERIALS?

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Trace analysis (*i.e.* the analysis of analytes in concentration low enough to cause difficulty, generally under 1 ppm) albeit very challenging, has shown in the last years a tremendous growth, prompted by the urgent need of many International Organizations (EPA, U.S. FDA, EFSA and WHO) looking for new analytical techniques for the detection of several molecules in different and increasingly more complex matrices [1].
The determination of trace analytes requires reliable and robust analytical methods characterized by high level of sensitivity, precision, accuracy, selectivity and specificity. Among different analytical techniques suitable for this purpose, electroanalysis, particularly pulsed methods, seems to be promising independent alternative in terms of very high precision, accuracy and sensitivity, simplicity of use, portability, easy automation and possibility of on-line and on-site monitoring, without sample pre-treatments and low costs.
These methods are no more confined to the detection of inorganic species and have been already and successfully employed for the determination of organic compounds and environmental carcinogens, as the Jirí Barek UNESCO Laboratory of Environmental Electrochemistry and the Trace Element Satellite Centre have amply demonstrated in the last decades [2-4].
In this context, recent technological developments have enhanced the chances of progress and growth of electroanalitycal methodologies for organic pollutants detection with the use of electrodes modified by nanomaterials (carbon nanotubes, metal nanoparticles, semiconductor nanoparticles) and/or biological probe (enzymes). The resulting sensors and biosensors were applied to the detection of furans, benzidines, alcohols, organic chlorides and trihalomethanes [5-8].

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ELECTROPOLYMERISATION AND ELECTROCHEMICAL BEHAVIOR OF POLYPYRROLE IN DEEP EUTECTIC SOLVENTS. AN EQCM STUDY

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Deep Eutectic Solvents (DESs) are rapidly emerging in the current literature as an interesting green alternative to conventional solvent systems [1,2]. DESs exhibit similar physico-chemical properties to the traditionally used ionic liquids (ILs) (e.g. nonreactive with water, non-volatile), while being much cheaper and more environmental-friendly.

In this report, we investigate the effects of electropolymerisation in DES on the properties of conducting polymers (CPs) films. As known, CPs are a class of materials that combine the advantages of organic polymers with the electrical and optical properties of metals or inorganic semiconductors. The applications of conjugated nanostructured polymers include sensors, actuators, flexible displays, solar cells, to name a few [3,4]. Subtle changes in the electropolymerisation conditions (solvent, supporting electrolyte, applied potential) bring about variations on the electrical (resistivity and capacitance), optical (absorptivity, luminescence, etc.), and mechanical properties of the CP films deposited on the electrode surface. In particular, the electrolyte used in the preparation and applications of the electroactive polymer has a significant influence on all the properties of the polymer. In this context, DESs represent a particularly interesting polymerization media, acting as solvent and electrolyte in one.

In this study we investigate the possibility to electropolymerise a common conducting polymer precursor, namely pyrrole (Py), in pure DES, without the addition of any other species, except for the monomer. The quaternary salt was choline chloride (Ch^+Cl^-) and the hydrogen bond donor was ethylene glycol (EG); this formulation is referred to as 'ethaline'. The deposition and growth of PPy films from ethaline and their electrochemical behaviour has been followed using electrochemical quartz crystal microbalance (EQCM) in order to collect important indications about the dynamics of ions transfer. In particular, at variance with what usually reported in literature for other CPs [5], the gravimetric measurements during doping-dedping processes of PPy in DES suggests the dominance of transfer of anions. We also demonstrate that the deposition process chosen leads to very reproducible results in terms of electrochemical charge-discharge processes and mass changes. Moreover, PPy films grown in DES show comparable electroactivity to those grown in H_2O, but the morphology of the surface is characterized by a higher degree of smoothness.

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DETERMINATION OF COLOUR INDEX AND POLYPHENOL CONTENT IN WINES THROUGH PEDOT MODIFIED ELECTRODES: A PRELIMINARY STUDY

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Phenolic compounds are a class of naturally occurring compounds widely distributed in nature, which are essential for the growth and reproduction of plants as well as for the protection against pathogens. Polyphenols are present in grapes and consequently in wines, but they can be also produced by yeast metabolism and extracted from the oak barrels in which wine is stored. The polyphenol content contributes substantially to the quality of wines and affects their color, stability, and aging behavior. Furthermore, the determination of this group of compounds can help to identify variants on type and differences in winemaking and maturation processes. Hence, it is of great importance to characterize a wine by its total phenolic content (TPP) and by its color index (CI), which is determined by the presence of a specific class of polyphenols, i.e. anthocyanin. The term 'total phenolic content' refers to the TPP amount, as measured obtained by spectrophotometric methods, especially the so-called Folin-Ciolcateu method, which are based on the reaction of phenols with a colorimetric reagent, thus inducing absorption in the visible region of the spectrum. On the other hand, high-performance liquid chromatography is used in the quantification and identification of individual polyphenolic substances, but it is not an easy, rapid or cheap approach. Therefore, the setup of new devices and methods for reliable analysis of TPP content represents an emerging topic, to which electrochemistry can give important contributions.

In this presentation, we show the first results collected by using poly-ethylenedioxythiophene (PEDOT) modified electrodes for the estimation of the TPP content and of the CI in different samples of wines. PEDOT-coated electrodes offer several advantages, among which the minimization of the fouling effects of the electrode surface due to polyphenols oxidation, usual for conventional electrode materials. Moreover, it works properly in aqueous media, which makes it attractive for direct analyses of food matrices, as already demonstrated in our previous studies and in Refs. [1,2]. In the preliminary part of this study, differential pulse voltammetry (DPV) and spectrophotometric measurements have been performed in model wine solutions containing different amounts of red grape skin extract powder (oenocyanin - EC). PEDOT-modified electrodes used in properly diluted wine solutions, buffered at different pH values give rise to repeatable DPV signals in which a well-defined current peak related to the EC content is detectable. A good correlation is found between the current intensity and the TPP content, as well as with the CI measured by spectrophotometric methods.

In the second part of the study, the correlation curves previously cited have been used for the determination of the TPP content and of CI of commercial wines. The comparison between the TPP and CI values obtained by the electrochemical and by the spectrophotometric methods is definitely satisfactory, giving sound reasons to go forward in this study.

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FUSION OF HYPERSPECTRAL IMAGING DATA FOR THE CLASSIFICATION OF GREEN COFFEE SAMPLES

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Data reduction and data fusion are two key issues in the analysis of large amounts of complex data, like those that can be obtained when using advanced sensing techniques. In this work, we have applied both these approaches to the analysis of datasets of complex 3D data, i.e., hyperspectral images acquired in the NIR range (900-1700 nm) for the classification of green coffee samples into *Arabica* and *Robusta* varieties.
The collected hyperspectral images were converted into three different kinds of signals: 1) average spectra (AS, 150 points long signals), 2) single space hyperspectrograms (SSH, 1200 points long signals) and 3) common space hyperspectrograms (CSH, 1050 points long signals). The hyperspectrograms[1] are built by compressing the useful information contained in each hyperspectral image into a signal composed by the frequency distribution curves of quantities calculated by Principal Component Analysis (PCA); a single PCA model for each image is used for SSH, while CSH is based on a common PCA model for all the images. This procedure allows to compress the information conveyed by the hyperspectral images, maintaining at the same time both spatial- and spectral-related features. Partial Least Squares-Discriminant Analysis (PLS-DA) was used as classification method firstly on single AS, SSH and CSH datasets of signals, then on fused data. Data fusion was performed both at the low level (Low-L), i.e., by simply merging the datasets (Figure1), and at the mid level (Mid-L), i.e., by merging the first 20 PC scores obtained by performing PCA on the different datasets[2]. Table 1 reports the classification performance, expressed in terms of efficiency in calibration (EFF_{CAL}), cross-validation(EFF_{CV}) and prediction (EFF_{PRED}), of the models obtained using both the separate approaches and the fused data

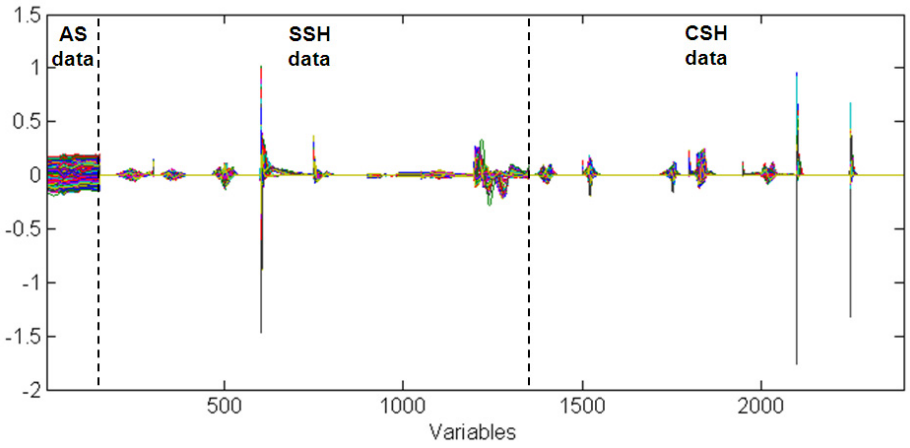

Figure 1. Block-scaled data fused at the low level.

	AS	SSH	CSH	Low-L data fusion	Mid-L data fusion
EFF_{CAL}	100 %	98.5 %	100 %	100 %	100 %
EFF_{CV}	99.5 %	93.7 %	93.8 %	94.2 %	97.6 %
EFF_{PRED}	91.3 %	90.3 %	100 %	95.8 %	98.6 %

Table 1. Results of PLS-DA models for *Arabica*/*Robusta* classification.

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P - 15

SENSING TOXICANTS IN MARINE WATERS MAKES SENSE USING BIOSENSORS

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Co-financed by the EU under "The Ocean of Tomorrow" programme, the SMS project promotes the development of novel sensing devices for marine environmental protection. The overriding goal of the project is to develop a novel automated networked system that will enable real-time in-situ monitoring of marine water chemical and ecological status in coastal areas by the detection of a series of contaminants. In this context, we are developing optical and electrochemical biosensors to detect four marine algal toxins: Saxitoxin (STX), Palytoxin (PTX), Okadaic Acid (OA) and Domoic Acid (DA), present at ultratraces levels in seawater.

Regarding STX, a low molecular weight neurotoxin mainly produced by certain marine *Dinoflagellates* and responsible for causing paralytic shellfish poisoning, we have developed an aptamer-based optical biosensors. In fact, optically labelled saxitoxin-binding aptamer can signal the target presence through a binding-induced conformational change which brings the fluorophore close to the quencher thus decreasing the fluorescence signal. The proposed method is simple, high specific and selective even if a preconcentration step is needed. Alternatively, we are also working on a flow-injection immunoassay (FI-IA) method with amperometric or colorimetric detection for saxitoxin determination. This system combines the rapidity and reproducibility of the flow-injection technique with the high selectivity and sensitivity of immunochemical reactions.

Regarding OA, a lipophilic marine toxin produced by *Dinophysis* and *Prorocentrum* which is responsible for causing diarrheic shellfish poisoning, we have developed a colorimetric assay based on the inhibition of protein phosphatase type 2A (PP2A) by the toxin.. We propose a colorimetric assay in which the activity of PP2A is determined by measuring the rate of color production from the production of yellow p-nitrophenol using p-nitrophenyl phosphate as the substrate.

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Riferimenti

1. S.M. Handy, et all., *Toxicon* 61 (2013) 30-37

P - 16

AN ELECTROCHEMICAL NITRATE SENSOR BASED ON Cu NANOWIRE ELECTRODES

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Ensembles of copper nanowire electrodes (CuWNEEs) are prepared via template electrodeposition in track-etched polycarbonate (PC) membranes [1]. Three different preparation methods are compared which differ for the way used to contact the PC membrane with a flat disk Cu electrode used as supporting material. The best results in terms of sensor durability and reproducibility are achieved by pre-sputtering a thin gold film on the templating membrane and attaching it to a supporting electrode by exploiting the adhesion property and ionic conductivity of a thin Nafion interlayer. SEM-EDS analyses together with double layer charging currents measurements indicate that the arrays are formed by copper nanowires with 400 nm diameter, 10 µm length distributed with a spatial density of 1×10^8 nanowires/cm^2 (Figure 1). The voltammetric reduction of nitrate at CuWNEEs is characterized by a well-resolved cathodic peak at approximately −0.680 V vs Ag/AgCl, whose current scales linearly with the nitrate concentration in the 10–400 µM range. The limit of detection (LOD) achieved by simple linear sweep voltammetry is in the 1.7–3.0 µM range, depending on the CuWNEE preparation method, such LOD values being among the lowest reported in the literature [2-4]. Analytical results obtained with the CuWNEE sensor for nitrate analyses in mineral water samples compare satisfactorily with those achieved by standard chromatographic or spectroscopic methods. Measurements to determine nitrate in cured meat samples with the CuWNEEs are in progress.

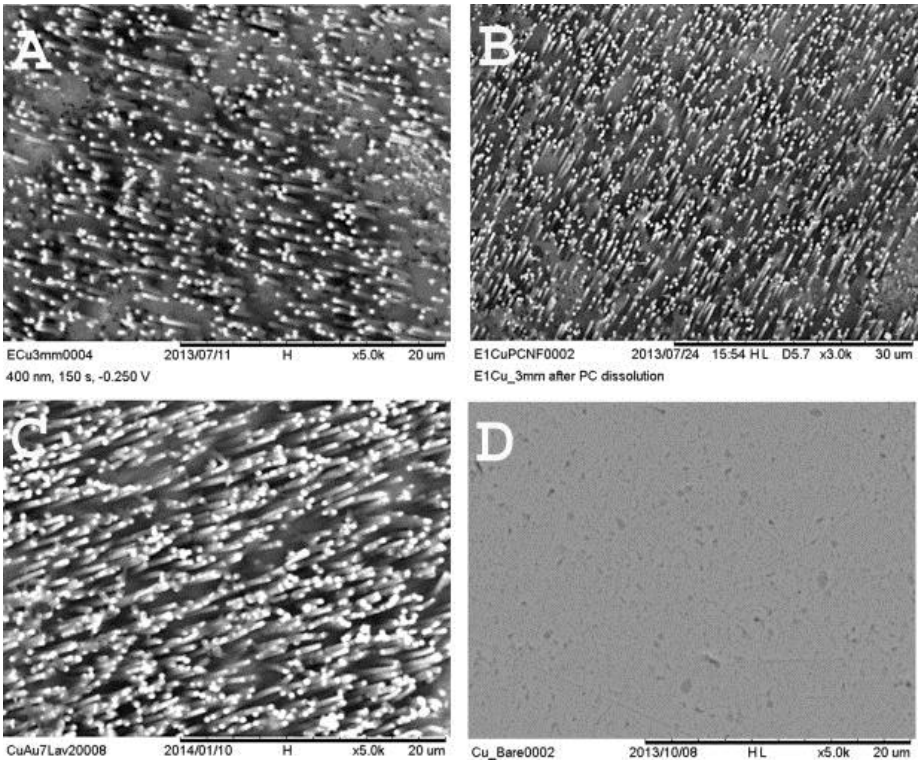

Figure 1. SEM images of CuWNEEs, prepared with different methods: (A) adhesion between PC and flat Cu by the pressure furnished by melamine foam, (B) adhesion by using a Nafion interlayer used as polyelectrolytic glue; (C) Nafion interlayer contacting a PC membrane pre-sputtered with gold , (D) comparison with a flat copper electrode.

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TOTAL ANTIOXIDANT CAPACITY AND POLYPHENOL CONCENTRATION OF SEVERAL PHARMACEUTICAL INTEGRATORS, MEASURED AND COMPARED USING TWO DIFFERENT ENZYME SENSORS

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Increasing interest has been aroused in recent years in the concept of antioxidant capacity linked to numerous natural plant products. Nevertheless, while the antioxidant capacity of the principal vegetal products (including fruit) is now sufficiently well known through the publication of numerous reports on the subject (some of which by the authors of the present communication), little or nothing is actually known from the experimental standpoint about the antioxidant capacity of many food supplements, most of which have appeared on the market recently and can be found in drugstores side by side with actual pharmaceutical products. Furthermore, although numerous methods are described in the literature to measure antioxidant capacity, the situation has become complicated ever since the United States Department of Agriculture [1] severely criticized and also withdrew from its catalogue the principal and widely known ORAC fluorimetric method [2,3]. Numerous workers have therefore recently gone back to using the polyphenol content of products as a measure of antioxidant properties rather than use methods based for instance on Hydrogen Atom Transfer (HAT), such as ORAC.

In recent years our research team has developed an original biosensor method based on superoxide dismutase for the purpose of measuring total antioxidant capacity (TAC) in many vegetal matrixes. This new method has been found to correlate highly with the ORAC method and with the classical spectrophotometric methods of the Electron Transfer (ET) type [4]. In the present research work this biosensor method has been used to determine the TAC of eight different food supplements available in drugstores and advertised above all as having antioxidant properties that can act as radical scavengers, that is, as capable of combating the oxidative stress due to the ROS. At the same time, in the same products, total polyphenol concentration, which is deemed to be one of the main and most effective radical scavengers of plant origin, was measured using a classical enzymatic-amperometric tyrosinase sensor [5]. This sensor has for some time been used for this purpose by our team as well as by other workers to measure polyphenol level in many real matrixes. The results of this research have without doubt led to the clarification of ideas on the true total antioxidant capacity of food supplements represented by those more readily available in drugstores and used as radical scavengers which are believed capable of combating the oxidative stress caused by often very serious pathologies. It has also been shown that TAC values and polyphenol concentration do not always correlate closely not only because of the presence of other non phenolic molecules that also have an antioxidant capacity in several of the products tested but also due to the different antioxidant capacity of the various polyphenols present. In addition, account must be taken also of the difficulty of total solubilizing and thus of effectively determining all the polyphenols contained in the real matrixes tested. Moreover, the solubilization of a product must always be considered as a prerequisite for it to be assimilated by the body.

One conclusion that may therefore be drawn from this research is that the determination of polyphenol concentration alone cannot always be taken as a completely reliable indicator of the total antioxidant capacity of real matrixes, particularly those represented by food-pharmaceutical supplements. On the latter point, however, further research is necessary and we are already working in this direction.

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BREATH FIGURES AS A VERSATILE TEMPLATING METHOD FOR INTERPENETRATED POLYMERS ELECTRODES

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This work describes the use of breath figures as a templating method for the fabrication of self-assembled interpenetrated polymers structures to assemble multipurpose biosensors. For the fabrication of good biosensors, two factors are essential, signal amplification and efficient enzyme coupling. So we proposed to use poly (3,4-ethylenedioxythiophene)–polystyrene sulfonic acid (PEDOT–PSS) electrosynthesized in a carboxylated polyurethane acid (PEUA). The breath figures are obtained simply by the fog created exhaling onto a cold surface where the polymer solution is casted. Once dried this surface appears as a templating membrane, fig.1. PEUA has the proper functional groups to link enzymes and other molecule. In a second step, the PEDOT-PSS was electrosynthesized into the pores of PEUA giving to the interpenetrated material. Finally, we used the glucose-oxidase enzyme coupled via the largely used EDC, NHS protocol. The presence of PEDOT allows improving the signal quality of the electrocatalytic H_2O_2 reaction in presence of glucose. Here an impedimetric biosensor based on electronic transfer between immobilized glucose-oxidase, which catalyse the reduction of hydrogen peroxide. The EIS spectra are showed in fig.2. The diameter of semicircles on the real axis represents the charge transfer resistance of the H_2O_2 reaction on the electrode.

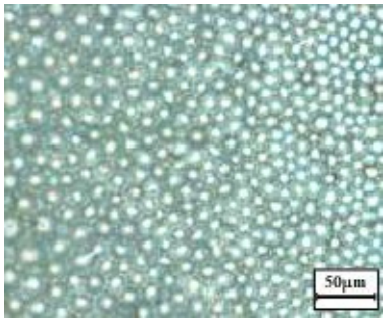

Fig.1 SEM images for PEUA breath figures**.**

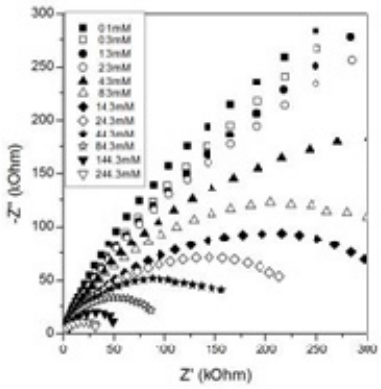

Fig.2 EIS spectra for H_2O_2 (0.1-240 mM), in PB pH=7 in OCV conditions.

COMPETITIVE IMMUNOSENSOR IMPLEMENTED ON GOLD NANOPARTICLES-MODIFIED SCREEN PRINTED CARBON ELECTRODES IN QUALITY CONTROL AND SAFETY MANAGEMENT OF "GLUTEN FREE" DECLARED FOODS

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Celiac disease is an immune-mediated enteropathy that occurs in genetically predisposed individuals as a result of gluten ingestion. Accordingly, gluten cross-contamination of "gluten-free"-declared foods is an emerging problem requiring the availability of rapid, highly sensitive and reliable analytical screening methods. In fact, for such foods the directives currently in force involve a maximum limit of 20 mg/kg of gluten [1], corresponding to about 10 mg/kg of gliadins, proteins belonging to the class of prolamines. As a part of our research programme on the development of innovative immunosensors aimed to direct detection of specific biomarkers for early diagnosis of celiac disease [2, 3], the present study concerns the development of an amperometric competitive immunosensor based on the immobilization of gliadin on the surface of gold nanoparticles-modified carbon screen printed electrodes (Figure 1) to be applied for conformity assessment of gluten-free declared foods. Considering the unavailability of reliable and certified standard materials for gliadin quantification, a bottleneck of the study was the set-up of the proper experimental conditions for standardization of gliadin extracts and sample treatment in order to achieve exhaustive extraction and avoiding matrix effects on the immunoassay. An optimization procedure, based on experimental design, was carried out to set the optimal concentrations of gliadin and anti-gliadin antibody, leading to a limit of detection and a dynamic response range analytically useful for the quality control of "gluten-free" foods.

Validation studies aimed to assess the suitability of the immunosensor in processed food matrices, which require also the use of reducing agents for proper sample treatment [4], are currently in progress. A commercial ELISA kit based on "sandwich" protocol was used as reference method.

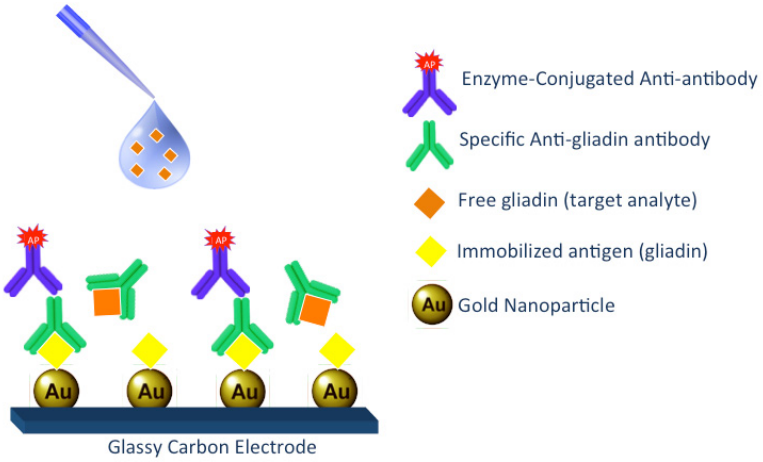

Figure 1. Schematic depiction of the set-up and working principle of the amperometric immunosensor

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ZnO NANO-TETRAPODS INTEGRATION ONTO A MICRO HOT PLATE STRUCTURE FOR GAS SENSING

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ZnO nanostructures are widely employed in micro and nano-devices due to their potential and peculiar properties, in different application fields, such as light emitting or laser diodes, field effect transistors, piezoelectric devices and many others. The MEMS technology offers a powerful tool to obtain low cost high efficiency and miniaturised devices. A nanostructures top down integration approach enables VLSI production scaling up since, in principle, more compatible with standard IC fabrication. In this view a novel gas MEMS based sensor that integrates a nanostructured ZnO film has a great potential in this field [1,2].
The presented device is based onto a micro hot plate membrane with Pt resistors for heating and temperature feedback, and Au electrodes for ZnO nanostructures contacting. The sensing principle is based on the resistance variation of the ZnO layer at specific temperatures due to an oxidation reaction of the target gas at its surface. As demonstrated [3], a ZnO tetrapods layer allows for reaching extremely high sensitivity thanks to the nanostructure conformation. The possibility to embed such nanotetrapods on a thin membrane will potentially increase the sensing performance, and speed up the response to obtain a gas detection sensitivity at the ppb scale with a much faster response than that of standard sensors [4].
A double-sided process via bulk micromachining was designed to fabricate the devices. Final ZnO tetrapods integration onto the active area between the electrodes was performed.
An exhaustive electrical and functional characterization is under development using a customized setup consisting of: Bronkhorst El-Flow thermal mass flow-controllers, instrument-grade stainless steel lines and a 150 cm^3 test cell, where temperature and relative humidity are continuously monitored by a Pt 1000 temperature sensor and a Honeywell calibrated humidity sensor. The proper mixing flows of synthetic dry air and water vapour saturated air with certified mixtures of diluted testing gases in air ensure an accurate humidity and gas concentration, thus allowing for extremely low concentrations control.

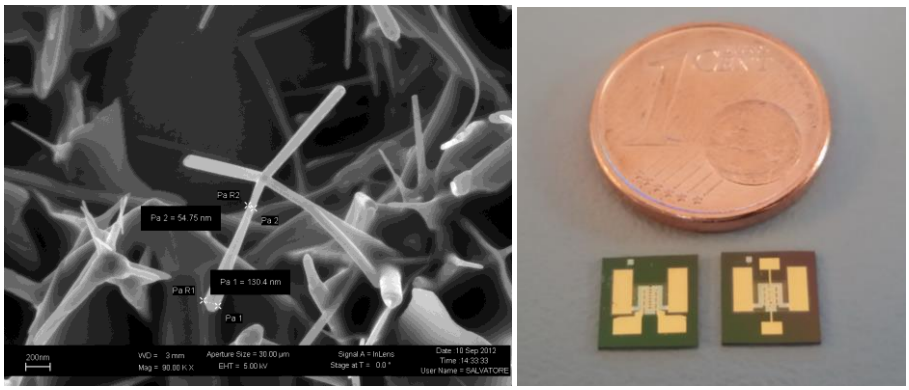

Figure 1. ZnO tetrapods (left) and micro hot plate gas sensors prototypes (right)

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DRUG-INDUCED CELLULAR DEATH DYNAMICS MONITORED BY ORGANIC ELECTROCHEMICAL TRANSISTORS

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Monitoring cell stress and death induced by drug treatments is an issue of great relevance, in particular impacting on toxicology, pharmacology, and therapeutics.[1] Nowadays several methods are used for cell viability detection, providing for high throughput results, reliability and sensitivity.[2] Nevertheless, they often require expensive laboratory equipments or biological kits, as well as specialized expertise and, in general, do not give real time monitoring. This has generated an increasing demand for easier, faster and portable tools for cell viability screening. A successful example of biosensors for toxicology and diagnostic monitoring are Organic Electrochemical Transistors (OECTs) based on conducting polymers, endowed by operation in aqueous environment, low operating voltages and ion-to-electron transduction.[3-8] Here, we present a sensitive and disposable OECT-based sensor for *in-vitro* monitoring of cellular dynamics, including stress and death. A549 adenocarcinoma cell line was cultivated on the micro-porous membrane of Transwell supports, that were directly integrated in the OECT. Cell response was investigated under the effect of the chemoterapic Doxorubicin, by monitoring both the time-evolution of cell death upon exposure to a fixed dose of drug, and the effect of different dose of drug monitored at a fixed time. The disruption of the cell layer caused by the drug effect could be effectively detected by our device, allowing to relate our signals alternatively to early apoptosis at low doses of drug or late apoptosis and necrosis for higher drug exposures. Results were interpreted by modelling the Twell-OECT device and validated by correlating the device response with traditional methods for cell viability/death assessment.
Our Twell-OECT represents a viable way to directly monitor cell death dynamics with cost effectiveness, portability and ease of use, paving the way toward point-of-care diagnostics.

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ZnO NANOTETRAPODS AND THEIR FUNCTIONALIZATION TO IMPROVE METAL-OXIDE BASED GAS SENSORS

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Zinc oxide (ZnO) is one of the most exploited semiconducting oxides for the fabrication of low-cost chemoresistive gas sensors. Being mainly based on nanocrystalline films, however, commercial sensors are generally subject to response drift in time because of sintering issues at the typical working temperature of 300-400°C.

Novel gas sensors have been produced by authors by using a different kind of nanostructure, i.e. nanotetrapods (Fig. 1). The highly porous network formed by these nano-sized single crystals, while preserving an high surface to volume ratio, is expected to reduce sintering effects during sensor life-time thanks to the small contact area between different nanostructures. ZnO nanotetrapod-based gas sensors have demonstrate to be highly sensitive towards a very large number of gases and volatile organic compounds, such as Co, NO2, H2S, ethanol, aldehydes, acetic acid, benzene, etc. [1-2].

Selectivity improvements, on the other side, have been studied by means of different surface functionalization on these nanostructures with different materials, such as other inorganic and organic semiconductors or metals [3-4]. Some examples, like for example selectivity improvements towards NO2 and acetic acid by means of a functionalization with chalcogenide nanoparticles or towards NO2 by means of a functionalization with phtalocyanine, will be discussed to demonstrate that these nanostructures are a solid base to develop new and improved family of gas sensors.

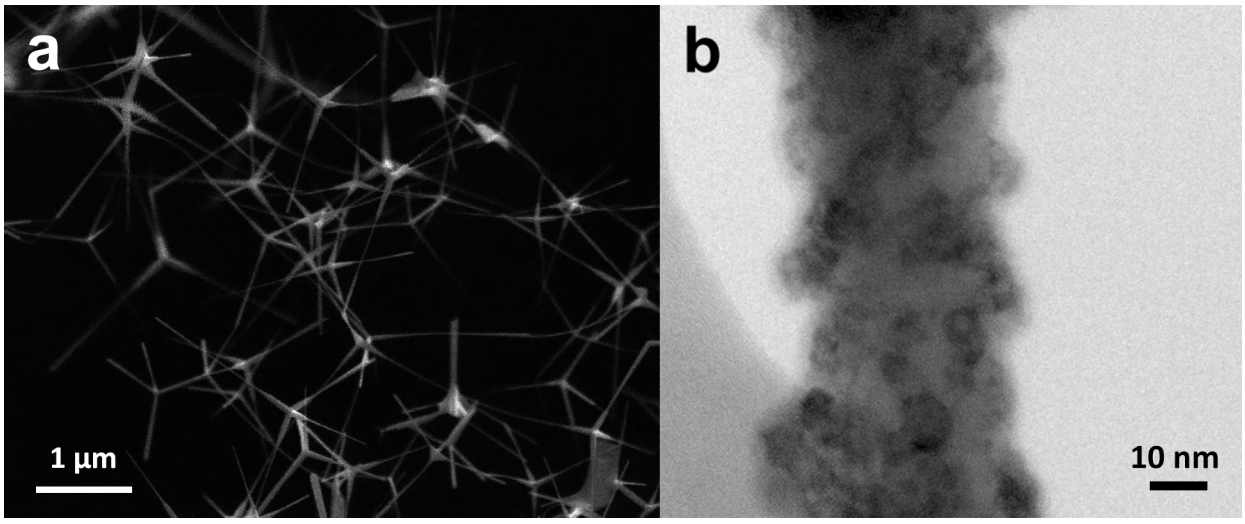

Figure 1. (a) SEM image of ZnO tetrapods used to fabricate gas sensors, (b) TEM image of a nanotetrapod leg functionalized with CdS nanoparticles.

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BIO-ORGANIC SENSING/MEMRISTIVE DEVICEs (BOSMDs): A DUAL FUNCTIONALITY TO SATISFY THE NEEDS OF BIOSENSORISTICS

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Mimicking the behaviour of living beings by means of man-made devices currently is a demanding challenge which is strongly pursued by the modern materials science[1]. This perspective is particularly felt as a new challenge to be taken on by scientists working in the field of Bioelectronics. Additionally, a growing interest aimed at demonstrating the feasibility of biodevices and bio-inspired systems[2] is becoming more and more established. Two kind of devices can meet most of requirements of Bioelectronics, i.e. memristors and Organic Electro-Chemical Transistors (OECTs)A memristor is essentially a device inherently endowed with memory while OECTs are the perfect candidate for biosensing applications, due to their ability to transduce very efficiently ionic signals into electronic ones. In this contribution, we show for the first time how memristors and OECTs can meet themselves upon merging them within a multifunctional device structure, hereinafter named Bio-Organic Sensing/Memristive Device, thanks to a living being, i.e. the Physarum Polycephalum Cell (PPC). PPC is an eukaryotic cell commonly studied due to its peculiar properties of "intelligence", "creativity" and "capacity of learning"[3].

The device layout is basically that of a conventional OECT whit an active channel made of poly (3,4-ethylenedioxythiophene) doped with poly(styrene sulfonate) (PEDOT:PSS), a stable and highly biocompatible p-type semiconductive polymer. In order to define a conducting channel, the as-patterned polymeric film is equipped with injecting electrodes, the Source and Drain, and interfaced with the PPC. The device properties have been electrically tested by using a series of different gate electrodes (Ag, Au and Pt) immersed in the PPC body, in order to check the standard response in the transistor mode (usually strongly depending on the choice of the gate electrode) by operating the device in a three-terminal configuration (PPC-OECT). The memristive like response has been checked by interdicting the drain electrode, so that the device is biased along a two-terminal configuration. The memiristive response, quite well classified within the Chua's generalized model for memristors[4], is uniquely attributed to the PEDOT:PSS/PPC interface, due to the specificity of the redox reactions induced in the analysed PEDOT:PSS/PPC/Gate electrode structures. The PEDOT:PSS/PPC interface has been demonstrated to be uniquely suitable to produce bioelectronics actions through a bifunctional transistor/memristor response. Furthermore, the PPC-OECT offers the opportunity to implement a biosensor-like operation through the direct monitoring of the internal cellular bioactivities and the environmental interactions of cells.

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COMPARATIVE INVESTIGATION BY IMPEDANCE SPECTROSCOPY OF GAS SENSORS BASED ON ZNO NANOTETRAPODS AND POROUS NANOSHEETS

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ZnO nanostructures (nanorods, nanowires, nanobelts, nanotetrapods and so on) have demonstrated great potential in gas sensing, owing to their small size, high-density surface active sites, and large surface-to-volume ratios. Layers made of these nanostructure show remarkable changes in the electrical conductivity upon chemical interaction of gas molecules with the semiconductor surface. However, even if some studies have been carried out to identify the mechanisms that relate the gas absorption to the electrical transport in nanostructured films[1], many aspects remain not well understood and a general model of the conduction in these systems is still lacking.
In this work, ZnO nanotetrapods (TPs) growth by vapour phase process [2] have been used as active layer in gas sensors on alumina substrates with a Pt heating element. The sensor response to different gases has been investigated by impedance spectroscopy measurements. Aiming to a deeper understanding of the charge transport mechanisms in these nanostructured films the rsults obtained are compared with those achieved by using mesoporous ZnO nanosheets (NSs) obtained from hybrid organic-inorganic precursor nanostructures [3] as the sensor active layer. It is shown that, in spite of the huge differences in the shape and dimension of the different nanostructures, the same equivalent circuit provides an excellent description of the behavior of both the TPs-based and the NSs-based sensors, in all the atmospheres considered.

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DIMENSIONALITY AND SIZE EFFECTS ON NANOSTRUCTURED METAL OXIDE GAS SENSORS

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Semiconductor metal oxide gas sensors are being widely investigated. Nanostructures are better candidate than their thick or thin film counterpart, mainly for their huge surface-to-volume ratio, which enhances the gas response. The use of single nanostructures in the fabrication of sensing devices allows to study their gas sensing properties in better detail, without averaging effects on several different structures and to explore the properties of the nanostructure itself.
As the sensing part of the material is the surface, a thinner nanostructure should show higher gas response. Such behaviour, intuitively following the space charge model, has been confirmed in many works, but still few are the reports on the diameter-dependence of nanowires gas response.
We present the growth and characterization of tin oxide single nanowires with different diameters, used as nitrogen dioxide sensors. The effects of working temperature, gas concentration and especially nanowire diameter on the sensing devices performance are investigated [1].
All the devices work at low temperature with a limit of detection of few ppm. All the sensors demonstrate rapid detection, with very short response and recovery times and good recovery degree.

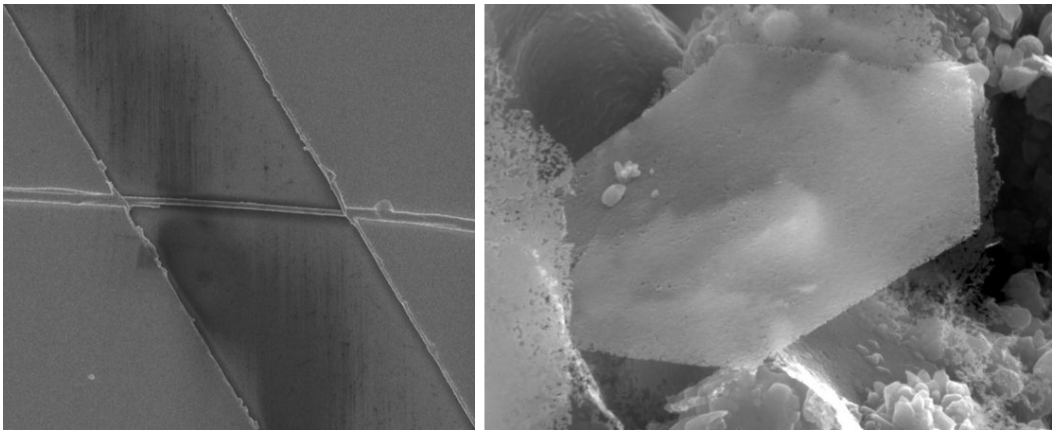

Figure 1. Single SnO_2 nanowire device (left); ZnO nanohexagon (right).

This study verifies the depletion layer model which is commonly used to explain the sensing mechanism of monocrystalline metal oxide nanowires.
Similar performance investigation and geometrical approximation are used to examine hydrogen and liquid petroleum gas (LPG) sensing properties of multiple one- and two-dimensional ZnO nanostructures. As expected, their larger cross section lowers their sensor response, but increases the intrinsic conductance, thus lowering the limit of detection of the sensing devices [2].

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GERMANIUM NANOWIRES AND CARBON NANOTUBES FOR EXPLOSIVES DETECTION

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Germanium nanowires can be funzionalized to detect TNT down to a detection limit of ~0.1 femtomolar (~$1x10^{-6}$ ppt). This can be achieved by the surface functionalization of Ge NW devices with an electron-rich amino-silane which binds the electron-deficient explosive molecules (e.g. TNT) through charge-transfer donor-acceptor interactions leading to charged TNT-amine complexes, thus causing sharp changes in the conductance of the electrical-sensing nanoelements (1). Germanium nanowires have been grown by Physical Vapor Transport using gold nanoparticles as catalyzer. Several hundredth μm long, few tens nanometer in diameter Ge nanowires (111) oriented with good size distribution were obtained.

Single-walled carbon nanotubes (SWNT) emit near-infrared (NIR) bandgap photoluminescence (PL) (2), which is highly responsive to its physical and chemical environment. SWNT are unique among nanoscale sensor platforms in their ability to detect the adsorption of as few as a single molecule of an analyte (3).

Carbon nanotubes (CNT) were deposited by using the aerosol chemical vapor deposition technique and Fe as catalyzer. This permitted to achieve Fe, Ni, Co filled or empty nanotubes with possibility of single and multiwall nanotubes and to obtain grams per run of carbon nanotubes.

The present work reports on the x-ray and electron microscopy characterization of Ge nanowires and carbon nanotubes to be used for sensors for explosives detection.

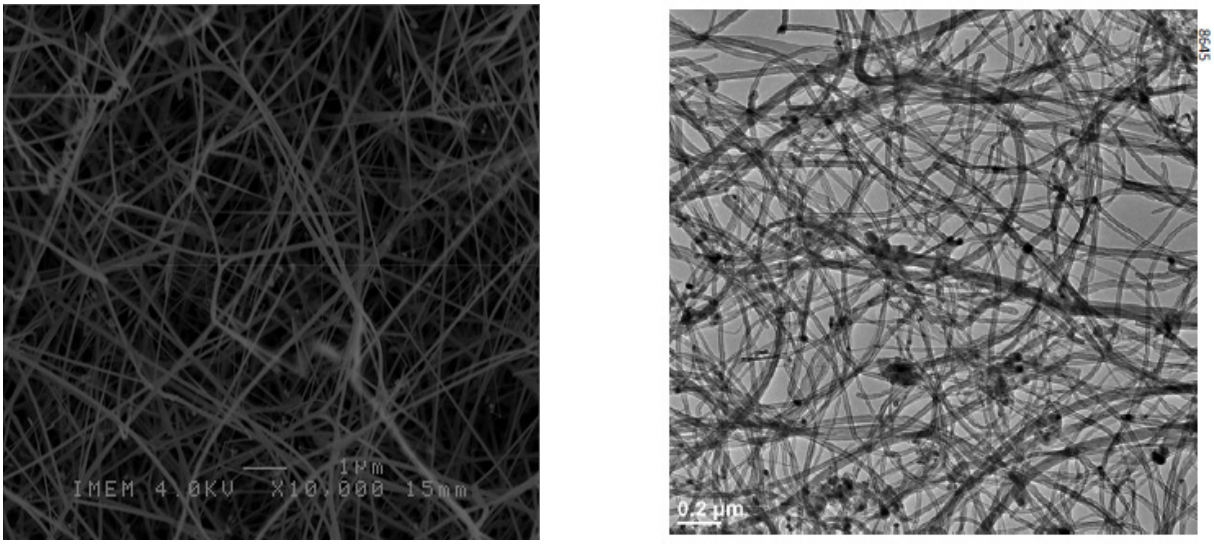

Figure 1. (a): Low magnification transmission electron micrographs showing germanium nanowires grown by the physical vapor transport method and (b) single and multiwalled CNTs

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ENHANCED SENSITIVITY TRANSDUCTORS BASED ON SILICA NANOWIRES GOLD DECORATED AND MAGNETO-PLASMONIC NANOSTRUCTURES

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Among all transduction methodologies reported in the field of solid state optical chemical sensors, the attention has been focused onto the optical sensing and biosensing characterization by using propagating and localized surface plasmon resonance (SPR) technique and its evolution towards active plasmonics. The research in this field is always oriented in the improvement of the sensing features in terms of sensitivity and limits of detection although quite smart results have been reached. To this purpose different strategies have been proposed to realize advanced materials for high sensitive plasmonic devices, as an example:

- Highly disordered system of silica nanowires (NWs) decorated with Au NPs present unique light trapping properties due to the combination of the highly diffusivity of transparent silica NWs, with the selective absorption resonances given by Au NPs.
- Activation of the transducer by the application of an oscillating magnetic field in a transversal configuration to improve the gas sensing performance of classical SPR sensors. In this case magneto-plasmonic nanostructures are used as transductors in which the combination of the magneto-optic (MO) effects of a magnetic material and the surface plasmon resonance (SPR) of metallic give rise to a new sensing probe. Since the MO effects are much localized, a very sharp curve can be obtained; as a consequence, small variations of the refractive index will induce large changes in the MO response, allowing to greatly improving the sensitivity of the MOSPR sensor.

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SCREEN-PRINTED ELECTRODE MODIFIED WITH CARBON BLACK NANOPARTICLES AND CHITOSAN AS A NOVEL PLATFORM FOR BIOSENSOR DEVELOPMENT

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In recent years, the use of nanomaterials in the biosensor sector has received particular attention owing their capability to improve the sensitivity and selectivity of the sensors. Among biosensors, enzyme-based sensors have demonstrated the suitability for applications in several fields such as clinical diagnostics, environmental monitoring, and food control.[1]
The immobilisation of the enzyme onto the working electrode surface is a key point in the development of a biosensor to ensure the functionality of the biomaterial, the accessibility towards target analytes, the close proximity between the bioreceptor and the transducer.[2]
The conjugation of biomaterials with carbon nanomaterials (such as carbon nanotube, carbon nanodots, Graphene) has been intensively studied because they can promote the electron transfer between enzyme and the electrode working surface.[3]
Among the carbon nanomaterials, the use of Carbon Black Nanoparticles (CBNPs) as modifying agent has reported considerable success in the electroanalysis and biosensing field; to this regard, our group has demonstrated the enhancement of the electrochemical performances of screen-printed electrode (SPE) modified with CBNPs in the detection of many analytes such as NADH, hydrogen peroxide, cysteine.[4]
In this work, we present a novel biocompatible platform for enzymatic biosensors based on the use of CBNPs and chitosan (Chit). For this purpose, the working electrode of a screen-printed sensor was modified by drop casting with CBNPs and Chit dispersion (CBchit-SPEs). Their electrochemical performances were investigated and compared with the unmodified SPE (Bare-SPE) using cyclic voltammetry (CV) in presence of ferro/ferri (cyanide) as electrochemical probe. The enzymes Laccase (Lacc) and Hydrogen Peroxidase (HRP) were immobilized by mixing directly these enzymes with the CBchit dispersion on the working electrode surface of the SPE, obtaining the CBchit/Enzyme-SPEs (CBchit/lacc-SPE and CBchit/HRP-SPE), and their responses towards hydroquinone (HQ) and caffeic acid (CA) were studied by cyclic voltammetry. The amperometric detection of the HQ in presence of hydrogen peroxide (H_2O_2) 1 mM at the applied potential of 0.0 V vs Ag/AgCl shows a linear range between 0.10 and 30 µM and LOD equal to 0.05 µM.
Using CBchit/Lacc-SPE, the CA was detected at the applied potential of 0.150 V vs Ag/AgCl reaching 1 µM as LOD and with a linear range from 1 to 10 µM.
The preliminary results show the suitability of CBchit-SPEs as platform for biosensor development with improved sensitivity and stability.

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