XXIIe RENCONTRE TRANSFRONTALIÈRE DE CAPTEURS ET BIOCAPTEURS

XXII TROBADA TRANSFRONTERERA DE SENSORS I BIOSENSORS

XXII TRANSFRONTIER MEETING SENSORS AND BIOSENSORS

Montpellier, France, 21-22 septembre 2017
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Electrochemical screening of transketolase inhibitors

Chloé Aymard, Bastien Doumèche, Loïc J. Blum

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Transketolase (TK), is a thiamine pyrophosphate (TPP) enzyme involved in the non-oxidative part of the pentose phosphate pathway. This enzyme plays a role in some pathologies such as cancer and Alzheimer’s disease. The TK is a therapeutic target of choice but at present, no inhibitor has been revealed. Since it appears essential to find inhibitors of this enzyme, a high-throughput electrochemical detection method was developed in our laboratory for screening of TK inhibitors. To this end, a system consisting of 96 electrodes screen-printed on a printed circuit board was designed. The development of this system as well as the first results obtained from the screening of the ICBMS chemical library will be presented.
FLUORESCENT PEPTIDE BIOSENSORS FOR PROBING KINASE ACTIVITIES: NEW TOOLS FOR CANCER DIAGNOSTICS AND DRUG DISCOVERY

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Keywords: Fluorescent Biosensor, Peptide, Protein Kinase Activity, Cancer

Cyclin-dependent kinases (CDK/cyclins) are heterodimeric kinases that play a central role in coordination of cell cycle progression and several essential biological processes [1]. These kinases are frequently hyperactivated in cancer and therefore constitute established cancer biomarkers and attractive pharmacological targets for anticancer therapeutics [2,3].

In order to propose sensitive and selective technologies for monitoring kinase activities, we have developed a toolbox of fluorescent peptide and protein biosensors, chemical probes which offer a straightforward means of sensing subtle alterations in kinase activity in vitro and in living cells, in tissue samples and tumour biopsies, and report on inhibition by kinase-specific drugs [4]. In particular, we have engineered a CDK4-specific biosensor which was implemented to quantify its activity in melanoma cell lines, xenografts and skin biopsies [5], and a CDK5/p25-specific biosensor which provides means of monitoring this kinase in neuronal cells and assessing its hyperactivation in neuronal disorders. More recently we have functionalized these fluorescent peptide biosensors onto carbon nanotubes to develop hybrid nanobiosensors with several new advantages. Taken together, these fluorescent biosensors constitute attractive tools for cancer diagnostics, for monitoring cancer progression and evaluating response to therapeutics, whilst also enabling development of sensitive assays for drug discovery [6,7].

References
Development of smart bandages by detection of metalloproteinases

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When a wound is healing, keratinocytes detach from the extracellular matrix and migrate to the wound bed using a provisional matrix.

There are many actors in the healing process such as cytokines, growth factors or enzymes like matrix metalloproteinases (MMP). They all work together to restore tissues and matrix.

There are two types of wounds: acute wounds which have a healing process with 4 « classical » steps and chronic wounds. This kind of wounds are known to be in a prolonged inflammatory stage.

One of the issues with non-healing chronic wounds like diabetic foot ulcers (DFU) or bedsores is the high level of matrix metalloproteinases because it is going to prevent the new collagen matrix to form thus stopping any healing process.

In this project, we chose to focalize our attention on diabetic foot ulcers, mainly because of its prevalence rates. Out of the 23 human MMPs we chose to investigate the level of MMP9 or gelatinase B.

One of the goals here is to be able to determine the level of this particular MMP and its importance in the healing process. To do so, we need to find a biological responsive element (BRE) able to recognize our MMP’s with high affinity.

We can divide this project in two big parts:

On the one hand, we need to find the biological responsive element using a phage display system. Once this step is complete, this BRE is tested to see the affinity rate with the gelatinase B.

On the other hand, in order to develop a sensor, we need to study the electrical response (variation of electric properties) of this BRE when with MMP-9 to do a passive radio frequency communication (RFID). To do so, a functionalization of BRE (biosensor) to the RFID tag constituted of porous alumina is necessary to show the possibility of a smart bandage.

This presentation will show all the researches that have been made for each parts, following by all the experiences and results that have been collected so far.
Key words: chronic wounds, healing, diabetic foot ulcers, MMPs (Matrix Metalloproteinases), Gelatinase, Biological Responsive Element (BRE), smart bandage, biosensor, RFID (Radio Frequency IDentification)
ELECTROCHEMICAL AND OPTICAL MAGNETO-ACTUATED PLATFORM FOR THE DETECTION OF NANOVESTICLES FROM BREAST CANCER CELLS

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Keywords: exosomes, biomarkers, magnetic particles.

The identification of novel biomarkers represents a worldwide challenge not only for the improvement of early diagnostics, but also for patient monitoring and for the evaluation of the efficiency of a therapeutic strategy. Exosomes are nano-sized and cup-shaped vesicles [1] (Figure 1A, inset A-B), which are currently under intensive study as potential diagnostic biomarkers for many health disorders, including cancer [2]. Therefore, this is a growing need for sensitive methods capable of accurately and specifically determining the concentration of exosomes. This work addresses the design of a quantitative and rapid method for total exosome counting from purified breast cancer cell culture supernatants based on magneto-actuated platforms with electrochemical readout, taking advantages of the improved bioanalytical features when integrating magnetic particles. Two different strategies were explored for the magnetic separation of exosomes. Briefly, based on i) the direct covalent immobilization on tosyl-activated magnetic particles or, instead, by ii) immunomagnetic separation on antiCD9, -CD24, -CD63, -CD81. The magneto electrochemical biosensor response of the exosomes counting was achieved with success (Figure 1B). Once the exosomes immobilized on the magnetic platforms, the direct labeling for electrochemical and optical readout using antiCD63 labeled with horseradish peroxidase (HRP) offers outstanding results in analytical performance. This proof-of-concept study as a rapid, cost-effective, and high-sample-throughput detection of exosome can potentially establish for promising applications in cancer diagnostics.

![Figure 1](image)

Figure 1. (A) Nanoparticle tracking analysis (NTA) on purified MCF-7 exosomes. (B) Electrochemical response of the exosomes count.

References
Development of a lithium sensor for its determination in blood

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The determination and control of some compounds present in blood can be useful to improve the wellness of people. In this sense, lithium ion is related to psychiatric diseases like bipolar disorders or schizophrenia. In order to carry out, by the patient, premature detection of anomalous lithium levels in blood, miniaturized analytical systems are needed which enable to obtain useful data in situ and in real time, or also follow the evolution of a compound through the time.

This work presents the development of miniaturized lithium ion selective electrodes (Li-ISEs) and an integrated potentiometric proof of concept. Analytical characteristics of three different lithium ionophores were evaluated to choose the one that offers better response features to perform analysis in blood. The ISEs, based on all-solid-state type, were fabricated following the polymer technology using Cyclic Olefin Copolymer (COC) as a substrate, with an epoxy-graphite conductive support and PVC membranes.[1]

In order to test and validate the potentiometric system, an artificial blood serum, prepared simulating the plasmatic medium, was determined using a proof of concept device fabricated in our research group integrating two Li-ISEs: one acting as a reference electrode and the other one as an indicator. Both of them were connected by a salt bridge. This new system configuration provides portability to the analytical device, showing comparable results to other potentiometric commercial equipments.

In the future, the goal is to design a disposable device for people who suffer disorders related to lithium concentration in blood, making possible its autonomous determination to take proper therapeutic decisions.

Electrochemical DNA sensor for *K. Armiger* based on 100% incorporation of Ferrocene labelled dATP and tailed primers.

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Electrochemical sensors have been exploited during the last decades for the detection of DNA due to their sensitivity, selectivity, miniaturization and inherent low cost. The advantages and disadvantages of the different approaches developed are discussed elsewhere (Drummond et al., 2003).

Here we purpose a novel DNA electrochemical sensor based on PCR amplification in presence of 100% ferrocene labelled dATP (or dA(EF)cTP), and the use of tailed primers.

The role of dA(EF)cTP is to label the resulting amplicon with a redox active moiety (ferrocene) for detection purposes. dA(EF)cTP was prepared by Sonogashira’s single-step aqueous-phase cross-coupling reactions of the iodo-dNTP precursors with ethynylferrocene and the product was purified and characterized before PCR reaction.

The role of tailed primers is to generate amplicons with single stranded DNA ends, that will be used to capture the amplicon on the surface of a modified gold electrode surface with the corresponding complementary capture probe.

Once the amplicon is captured on the electrode surface and the excess of reagents are washed away, the ferrocene linked to the amplicon can be measured directly without the need of any enzymatic system.

*Karlodinium Armiger*, a well-known toxic microalgae, was selected as model target but the strategy can be extended to other targets.

References

https://doi.org/10.1038/nbt873
COMPARING ELECTROCHEMICAL MAGNETO IMMUNOSENSING AND GENOSENSING FOR THE DETECTION OF CIRCULATING TUMOR CELLS

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Keywords: Immunomagnetic separation, ELISA, DNA Amplification, Biosensors, Breast Cancer, CTCs.

Cancer affects the daily life of millions of people worldwide, accounting for nearly 1 in 6 deaths in 2015. 8.2 million people die from cancer annually, while 14 million of new cases are reported. According to WHO, breast cancer is the top cancer in women both in the developed and the developing world, and the number of new cases is expected to rise by about 70% over the next 2 decades. The early and accurate detection of breast cancer as well as the risk of metastasis in small healthcare centers remains as the cornerstone of breast cancer control in order to improve breast cancer outcome and survival.

This work is intended to contribute in the development of Rapid Diagnostic Test (RDTs) for cancer diagnosis at point-of-care in low resource settings, taking breast cancer circulating tumor cells from MCF7 cellular line as a model. Two different strategies were designed: a magneto-actuated immunosensor for the quantification of the breast cancer cells and a magnetic genosensor for the detection of the PCR-amplified genetic material from the cells.

For that purpose, different commercial antibodies against specific epitopes of the cellular membrane were firstly studied by flow cytometry. Such antibodies were then covalently immobilized on magnetic particles to capture the tumor cells by immunomagnetic separation for the preconcentration of the cells from complex samples. The magneto genosensing approach is based on a double-tagging RT-PCR amplification of the RNA from the cells and the quantification of the amplicon by amperometry technique. Finally, the results of the two strategies were compared in terms of the analytical performance, showing promising features for being used as RDTs.
Development of an RPA-ELONA assay for the rapid detection and quantification of two Karlodinium species: K. veneficum and K. armiger

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Ebro Delta is affected by recurrent Karlodinium spp. blooms that cause fish mortalities. Specifically, two species with different level of toxicity have been distinguished in these events: K. veneficum and K. armiger. Current toxic microalgae monitoring is performed by optical microscopy, which is time-consuming and hinders rigorous microalgae identification. Therefore, rapid, accurate and in-situ platforms are highly needed to provide timely warnings.

With this purpose, we report the development of a recombinase polymerase amplification (RPA) combined with an enzyme-linked oligonucleotide assay (ELONA) to rapidly and specifically detect and quantify Karlodinium spp. and also discriminate between K. veneficum and K. armiger. The set of primers (two species-specific primers and one genus-specific primer) used for the amplification were designed within the ribosomal DNA and then modified by adding probe-complementary tails at the ends. For the ELONA assay, two distinct thiolated-capture probes were immobilized on maleimide microtitre plates, which specifically hybridize to their corresponding RPA amplicon. Finally, colorimetric detection was achieved via the addition of an HRP-reporting probe.

The qualitative detection of both Karlodinium species was achieved and showed high specificity within Karlodinium species and no interferences towards other microalgae genera. Calibration curves using synthetic ssDNA and genomic dsDNA were successfully constructed, demonstrating the feasibility of the approach. Afterwards, standard curves using ten-fold cell dilutions (from 10⁶ to 10⁴ cells) were obtained for K. veneficum and K. armiger. In this regard, a new, rapid and simple DNA extraction method was optimized and used for this purpose. Finally, the developed method was tested with spiked and naturally contaminated samples and results were compared to light microscopy counting and qPCR analysis. Good agreement was obtained among techniques, indicating the assay reported here is a promising, reliable and simple alternative tool for Karlodinium spp. monitoring.

Acknowledgments

The authors acknowledge financial support from the Ministerio de Economía y Competitividad (MINECO) through the BIO2014-56024-C2-2-R project. Anna Toldrà acknowledges scholarship from IRTA-URV-BANCO SANTANDER. We thank María Rey, Josep Fumadó and José Luis Costa for the technical help.
Multivariate standard addition applied to the determination of Tl(II) and In(III) in complex matrices by means of a sensor array

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Thallium and indium are relatively rare metals that do not have any essential biological role but have been shown to be highly toxic. Due to their unique chemical properties, the use of these metals has increased in the last years, particularly in medical procedures and electronics industry.

The simultaneous stripping voltammetric determination of Tl(II) and In(III) based on the use of traditional working electrodes is somewhat problematic due to their highly overlapped signals. More promising results have been reported using sensor arrays [1]. However, the typical calibration method used for sensor arrays does not allow the solving of matrix effects, preventing the analysis of complex samples. Therefore, alternative calibration strategies are required.

In this work, we report the simultaneous determination of Tl(II) and In(III) in tonic water using a sensor array formed by an ex-situ bismuth film deposited on a screen-printed carbon electrode and a screen-printed carbon nanofiber electrode chemically modified with selenocystine. In order to solve the matrix effects, a new calibration strategy based on the combination of the standard addition method and PLS calibration has been developed. This strategy provides good precision and trueness, with relative errors of 0.68% and 2.86% for Tl(II) and In(III) respectively.

Love wave devices coated with TiO2 porous sensitive layer for direct detection of small molecules

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The detection of low molecular weight targets is an important issue for many domains such as environment and health. In this communication, we describe an architecture which consists in a Love wave transducer and a porous sensitive layer functionalized for the direct detection of such small molecules. This architecture could allow real-time monitoring of water quality, with interesting features like being low cost, reliable and miniaturized.

Love wave transducers have already demonstrated their ability to detect various species in liquid medium in real time [1], [2]. A surface interaction between the acoustic Love wave, and a sensitive film which consist of an antibody layer specific to the target compound, leads to good results. However, these devices have some limitations for the detection of low molecular weight targets like toxins [3]. The porous property of the TiO2 film will provide a significant increase in the overall specific surface which can improve the sensitivity of the sensor as it has been demonstrated in a gaseous medium [4]. We present the first experiments and modelizations using a Finite element software which confirms that this approach could increase the sensitivity of the device 30 times better than a traditional one.


Biosensors for marine monitoring and research

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Society has great concern and expectation about the health of oceans and seas and their ability to continue providing resources and goods. The capacities of the marine ecosystems are limited and their exploitation is often not optimally managed. It is necessary to sustainably use aquatic living resources to maximise social and economic benefits/returns from oceans and seas. The monitoring of the sea can contribute to guarantee this sustainable management. Nevertheless, and because despite its charm, sea is a harsh working environment, the availability of tools to control parameters of interest is limited.

Biosensors provide many added advantages over traditional analysis methods. The high sensitivities, low limits of detection, short analysis times, simplicity of use even for non-skilled personnel, low cost, miniaturisation, automation and portability, make interesting their development for the subsequent implementation in seafood safety and public health protection programmes. The deployment of biosensors into the marine environment can contribute to manage shellfish production areas and aquaculture facilities, to understand phytoplankton population dynamics, to study marine toxin transfer within food webs, to identify areas at risk of fish and shellfish poisoning, to predict the state of coastal waters, and to evaluate possible impacts of the climate change, among others.

In the Marine Monitoring Subprogram of IRTA, we develop bioanalytical systems for the detection of analytes of interest in aquaculture applications: marine toxins (okadaic acid and derivatives, yessotoxins, azaspiracids, tetrodotoxins, and ciguatoxins), aquatic pathogens (oyster herpes virus), toxic microalgae (Karolodinium, Ostreopsis and Gambierdiscus) and biogenic amines (histamine, putrescine and cadaverine). Immobilisation strategies based on self-assembled monolayers with mono- and di-thiolated molecules, magnetic particles as immobilisation supports, signal amplifiers or analyte capturing material, electrode nanostructuration with diatom frustules, and isothermal DNA amplification techniques are examples of our recent efforts. The bioanalytical systems have been applied to the analysis of shellfish, puffer fish, microalgae cultures, seawater and even human body fluids.

Acknowledgments

The research leading to these results has received funding from the Ministerio de Economía, Industria y Competitividad through the SEASENSING (BIO2014-56024-C2-2-R) project, from the H2020 EU Framework Programme through the VIVALDI (H2020-SFS-678589) project, and from CERCA Programme / Generalitat de Catalunya.
Miniaturized analytical flow-system integrating pH-ISFET and gas-diffusion for monitoring free sulfur dioxide and acetic acid in wines

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High concentration of free SO\(_2\) or acetic acid in wines can induce diseases in people with food intolerances and is related to bacterial activity, deteriorating the wine flavor and smell. Therefore, the control of both acids during the ageing of the wine in barrels is critical for guaranteeing the quality of the final product. Currently, to control both parameters traditional methods using complex equipment and tedious methods are applied in external laboratories. Therefore corrective actions cannot be applied in-situ. In order to solve this problem, a miniaturized flow-system integrating a pH-ISFET and a gas-diffusion step for measuring SO\(_2\) and acetic acid is proposed in this work. The principle of determination is based on the acid/basic characteristics of the analytes and their volatile/gas state in the acidic form. This fact allows the separation of the analyte from the sample and its indirect detection by measuring the pH change in an acceptor solution. The pH measurement is carried out with a miniaturized pH-ISFET, device fabricated by microelectronic technology. The proposed flow-system is fabricated using poly methyl methacrylate (PMMA) and pressure sensitive adhesive (PSA) manufactured by CO\(_2\)-laser ablation. Since the system is planned to be placed in the ageing wine barrels, it needs some special requirements of miniaturization and reduction of reagents and sample consumption. For that reason the gas-diffusion chamber and the pH detection cell are joined in the same assembly (3 cm × 7 cm × 2 cm) as it is shown in Figure 1.

The hydrodynamic and chemical parameters of the flow-system were optimized in aqueous solutions for each analyte, studying their analytical performance in terms of sensitivity, limit of detection and linear range, fulfilling the requirements of the proposed application. Finally, the developed flow-system was applied to the control of the acetic acid and free SO\(_2\) in several samples of red, whites and rosé wines. The results obtained with the flow-system were compared with the standard official methods applied by the IRTA-INCAVI, showing a good agreement between them.

Acknowledgements: We acknowledge funding from the Spanish R & D National Program (MINECO, TEC2014-54449-C3-1-R04). P. G.-G. is grateful to MINECO, Spain, for the financial support through a research studentship of the FPI Program. The financial support from Sapere Corporation is also acknowledged.
Aptasensors, as applied for environmental and food safety to detect contaminants and toxins, has gained tremendous interests because of the promise of detection in a faster, simpler and cheaper manner compared to the traditional analytical methods. For these reasons, many research efforts were devoted to the development of new platforms that are particularly based on electrochemical techniques thanks to their cost-effectiveness, rapid response and higher sensitivity. Besides, it has been demonstrated that the integration of nanomaterials in biosensing platforms induce significant improvement in sensing metrics due to their unique proprieties such as electrocatalysis, biocompatibility and especially high specific surface. Recently, silicon nanoparticles have been used in biosensing because of their intrinsic advantages, among which we can mention their low cost, strong fluorescence, ultrahigh photostability and high capacitive power. However, this new nanomaterial is not yet well exploited in electrochemical biosensors.

In this work, we report a novel label-free aptasensor based on silicon nanoparticles designed for the detection of aflatoxin M1 (AFM1). Given that this nanomaterial stands out by its high capacitive power, we developed a highly sensitive transduction system based on changes in the capacitance derived from the measurements of electrochemical impedance spectroscopy response in non-faradic regime. The calculated capacitance real component $C'(F)$ at a fixed lowest frequency was found to correlate well with the increasing concentration of AFM1. The dynamic range reveals a linear sensing response from 20 to 250 pmol/L with a sensitivity of $0.19 \, \mu F.pM^{-1}.cm^{-2}$ and a limit of detection equals to 16.13 fmol/L. The platform was challenged against ochratoxin B and picrotoxin as potential interfering mycotoxins to reveal the capacitance changes are only induced to the presence of the analyte. Furthermore, the aptasensor was applied with success to detect very low concentrations of the mycotoxin in commercial pasteurized milk, though these results are to be confronted with to other analytical methods such HPLC.
Self-assembled monolayer-based immunoassays for early warning detection of diarrhetic shellfish poisoning toxins

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Among the several illnesses caused by contaminated bivalve molluscs, diarrhetic shellfish poisoning (DSP) is the most significant problem in regions with well-developed aquaculture activities in temperate seas. The DSP toxin group includes okadaic acid (OA) and its analogues dinophysistoxin-1 (DTX-1) and dinophysistoxin-2 (DTX-2), which are produced by dinoflagellates of the Dinophysis and Prorocentrum genera. The development of fast and low-cost methods to monitor the presence of these toxins in seawater samples in a rapid, easy and reliable way is required to facilitate important decisions to be taken before the contamination of shellfish. A self-assembled monolayer (SAM)-based immunoassay for the detection of OA and its derivatives is presented. The use of SAMs as building blocks in immunoassays enables to control the orientation, distribution and spacing of the immunoepitopes while reducing non-specific interactions. In this work, monothiols (cysteamine) or dithiols (dithiol-alkane aromatic PEG6-NNH2) are used to covalently immobilise OA through their amine or hydrazide groups, respectively. The use of cysteamine provides a short-chain and packed monolayer, which would allow a high antigen immobilisation yield. In contrast, dithiols provide long-chain SAMs with a multivalent mechanism of interaction, providing more stable and spaced monolayers than monothiols and allowing an improved mobility and flexibility of the immobilised biomolecule at the recognition terminus. A competition step between free and immobilised OA for the anti-OA antibody is performed and calibration curves are obtained after the incubation with an HRP-labelled secondary antibody. Cross-reactivity factors for DTX-1 and DTX-2 are established and both SAM-based immunoassays are applied to the analysis of seawater samples collected from different points of two Catalan harbours (NW Mediterranean) and seawater samples collected periodically from the Galician Rias (Atlantic), as well as certified reference material of DSP-contaminated mussel. Toxin quantifications provided by the SAM-based immunoassays are compared with those provided by LC-MS/MS analysis and the certified values. The correlation between OA content and the presence of Dinophysis in seawater samples is investigated. Thus, the potential application of the SAM-based immunoassays as a highly valuable tool for both early warning in monitoring programs as well as research purposes is demonstrated.
Figure 1. Schematic representation of the SAM-based immunoassays for the detection of OA.

Acknowledgments
The research leading to these results has received funding from the Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA) through the PROMAQUA project (RTA2013-00096-00-00) and from the Ministerio de Economía, Industria y Competitividad through the SEASENSING (BIO2014-56024-C2-2-R) project. The authors acknowledge the shellfish monitoring program of Catalonia (Departament d’Agricultura, Ramaderia, Pesca, Alimentació i Medi Natural, Generalitat de Catalunya) and the Galician Monitoring Program (www.intecmar.org) for collecting and providing seawater samples and supply information on phytoplankton presence. The authors also acknowledge support from CERCA Programme / Generalitat de Catalunya. Sandra Leonardo and Anna Toldrà acknowledge IRTA – Universitat Rovira i Virgili – Banco Santander for their PhD grants (2013PIPF URV-IRTA-BS-01 and 2015PMF-PIPF-67).
Detection of Mycotoxins in Food by Advanced Sensors

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Abstract

Governments and international instances are trying to improve the food security system to prevent, reduce or avoid the increase of food borne diseases. This food risk is one of the major concerns for the humanity.

The contamination by mycotoxins is a threat to the health and life of humans and animals. Indeed, many of these mycotoxins have proved to be carcinogenic, teratogenic and mutagenic.

Our thesis topic focuses on the development of an electronic system allowing simple and rapid quantification of mycotoxins in food.

This system will implement the knowledge on the mycotoxins molecular sensors (aptamers), an electronic device that will link the information, the quantification and make it available to operators.

This work is carried out within the team of Dr Didier Montet, HDR who is the main supervisor of the student, whose team is highly specialized on the analysis of mycotoxins in food and the team of Prof. Brice Sorli (Co-supervisor) of the Electronics Institute of CNRS UMR 5214, where the study of the electrical response (variation of electrical properties) of this aptamer in the presence of mycotoxins in order to be able to make a pairing with a radio frequency
communication passive-type RFID. This device which is characterized by its low cost, its speed and a simple wireless information transmission.
Optical and electrochemical detection of toxic microalgae using thermal and isothermal amplification techniques

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ASSURED (Affordable; Sensitive; Specific; User-friendly; Rapid; Equipment-free; Delivered to those who need it) diagnostics is the benchmark for the development of biosensors. Although for simple metabolite and protein detection exist ASSURED biosensors, for molecular diagnostics (detection of specific DNA sequences) there is no platform. Yet, molecular diagnostics can significantly improve health care, food safety, usher the age of personalised medicine, and protect against bioterrorism.

We demonstrate elements that can lead to the ASSURED platform for molecular diagnostics. As example we use the molecular detection of toxic microalgae where field-based, real time detection has obvious advantages. We show thermal and isothermal amplification of chosen targets by Polymerase Chain Reaction (PCR) and Recombinase Polymerase Amplification (RPA) in liquid phase with further detection by gel electrophoresis, optical and electrochemical methods. In molecular diagnostics amplification of the target is necessary due to the low detection limit requirement in most of the applications. Isothermal amplification allows incorporation of molecular diagnostics in devices that can be used in the field because of low power requirements and no need for programming of thermal cycling.

The novelty of this work is the usage of electrochemically labelled dNTPs during amplification process. This approach allows us to significantly shorten the assay time. For our project we chose Aminophenyl-Labeled Nucleoside Triphosphate. We demonstrate 100% incorporation of the labels by both PCR and RPA with further detection.
Posters
SYNTHESIS AND CHARACTERIZATION OF CARBON DOTS FOR THE ANALYSIS OF HEAVY METALS

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Research for new automated and miniaturized analytical procedures and instrumentation for the continuous monitoring of chemical and biochemical parameters, for instance those affecting water quality, has acquired special importance in the last years. In this sense, Lab-on-a-chip devices are employed with this purpose due to their high integration and automation and that are capable of performing in situ measurements.

The use of nanoparticles with analytical purposes has demonstrated to improve the sensitivity and detection limits of optical methods. This work is based on the use of Carbon Dots as optical labels, for the rapid detection of water pollutants such as heavy metals. However, the synthesis of reproducible Carbon Dots needs further investigation in order to obtain nanoparticles with identical physical characteristics to acquire reliable and reproducible analytical measurements. The research group has proposed several microreactors for the synthesis process intensification. The idea is to be able to synthesize and use the Carbon Dots as optical labels for in situ monitoring without further purification.[1]

Carbon Dots usually exist in the size of less 10 nm, showing excellent properties such as ease of preparation, satisfactory fluorescent performance, low cytotoxicity and biocompatibility.[2] Their fluorescence emissions in particular have attracted increasing interest in recent years, replacing semiconductor Quantum Dots that possess certain limitations such as high toxicity due to the use of heavy metals in their production.[3]

In this work, the synthetized Carbon Dots allow the monitoring of mercuric ion in water with excellent sensitivity and selectivity. The fluorescence signals are on-line registered by a miniaturized optical detection system integrated in the microfluidic platform. Once optimized, the device is capable to detect up to 2 ppb of mercuric ion. Good reproducibility is also achieved. The presented results demonstrate the great potential of the combination of automated and miniaturized analytical microsystems with the use of nanoparticles for the monitoring of environmental pollutants.

Competitive direct electrochemical immunosensors for the detection of azaspiracids

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Azaspiracids (AZAs) are lipophilic marine toxins that originate from the phytoplankton Azadinium spp. and Amphidoma spp., which can accumulate in shellfish, leading to severe acute and suspected chronic toxicological effects on humans after the consumption of the AZA-contaminated shellfish. As AZAs are being reported from the coastal waters of an increasing number of countries on a global scale, the need for rapid, simple and cost-effective methods to detect these marine toxins and protect seafood consumer’s health is becoming evident. In this sense, electrochemical immunosensors can provide expedient solutions to the most contemporary demands of biochemical sensing of azaspiracids, providing high sensitivities, low cost, possibility of miniaturisation and automation and multiplexed analysis. However, a key point in the development of electrochemical immunosensors is the immobilisation of the antibody or antigen onto the transducer. In this work, two electrochemical immunosensors for the detection of azaspiracids are presented, which are obtained by means of different antibody immobilisation strategies. The first one, based on the affinity of the Protein G to bind to the Fc region of the antibody, allows an oriented and stable immobilisation of the antibody avoiding possible steric effects. The second one, which requires the biotinylation of the antibody, takes benefit from the almost irreversible avidin-biotin interaction, favouring the elution of the antigen from the antibody and making possible the immunosensor regeneration. Both immunosensors, based on a direct competition between the free AZA and an AZA-HRP conjugate for the binding sites of the antibody, provide similar analytical performances, allowing the detection of a wide range of AZA concentrations from below the regulatory limit (160 µg AZA-1 equiv./kg) to far in excess the threshold. The immunosensors are applied to the AZA quantification of 16 naturally contaminated mussel samples and results are compared with those obtained by LC-MS/MS analysis. The excellent correlation obtained with the reference method demonstrates the use of the immunosensors as both screening tool at the regulatory level and quantification analysis technique in a simple and reliable way. Their selectivity, robustness and cost-effectiveness provide valuable tools for the detection of all toxic AZA analogues suitable for end users in the field of food safety, leading to the implementation of compact and automated electrochemical biosensors for high throughput sample analysis in seafood safety monitoring programs.
Acknowledgments
The research leading to these results has received funding from the Ministerio de Economía, Industria y Competitividad through the SEASENSING (BIO2014-56024-C2-2-R) project and from the European Union’s Seventh Framework Programme (FP7/2007-2013) under the Sea Change strategy (PBA/AF/08/001(01) with the support of the Marine Institute and the Marine Research Sub-Programme of the National Development Plan 2007–2013, co-financed under the European Regional Development Fund. The authors acknowledge financial support from CERCA Programme / Generalitat de Catalunya. Sandra Leonardo acknowledges scholarship from IRTA-Universitat Rovira i Virgili-Banco Santander (2013PIPF URV-IRTA-BS-01).
TOPIC: Applications on environmental monitoring, medical, food and industrial fields.

Monitoring of the malolactic fermentation of wines using a miniaturized flow-system integrating bienzymatic amperometric biosensors

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During the malolactic fermentation (MLF) of red wines, the L-malic acid is mainly converted to L-lactic acid. The acid concentration along the process has a significant influence on the quality of the final wine, therefore the real-time monitoring of the MLF is necessary. The traditional methods applied at present do not meet this basic necessity because they are carried out off-line and do not offer the possibility to take actions in time. In order to solve it, a miniaturized and portable device is developed in this communication. The system is based on a silicon chip (9 mm x 11 mm) with four thin-film platinum electrodes in parallel: a 2.5 mm\textsuperscript{2} pseudo-reference electrode (p-RE), two 2.5 mm\textsuperscript{2} working electrodes (WE) and a 5 mm\textsuperscript{2} counter electrode (CE) (Figure 1a). The chip is integrated in a portable and robust poly (methyl methacrylate) (PMMA) and pressure-sensitive adhesive (PSA) structure formed by several individual layers (17 mm x 30 mm) allowing the positioning of the chip and its alignment with the fluidic reservoir and channels (Figure 1b). Both working electrodes are electro-modified with a three-dimensional polypyrrole membrane entrapping the reagents involved in the bienzymatic reactions used for the acids' determination \cite{1, 2}.

The developed microsystem is connected to a portable potentiostatic and its analytical response was recorded in a buffer solution containing the remaining reagents. The L-lactate biosensor shows a sensitivity of \(-173\pm8\) \(\mu\text{A} \text{M}^{-1} \text{cm}^{-2}\) \((r = 0.997, n = 7)\), a linear range (LR) from \(5\times10^{-6} \text{ M}\) to \(1\times10^{-5} \text{ M}\) and a limit of detection (LOD, according to the 3\textsuperscript{rd} IUPAC criterion) of \(3.2 \pm 0.3 \times 10^{-6} \text{ M}\). The L-malate biosensor has a sensitivity of \((5.53\pm0.6) \times 10^{-2} \text{ mA} \text{M}^{-1} \text{cm}^{-2}\) \((r = 0.997, n = 5)\), a LR from \(1\times10^{-7} \text{ M}\) to \(1\times10^{-6} \text{ M}\) and a LOD of \(6.7\pm0.2\times10^{-8} \text{ M}\). Besides, the long-term stability showed for both biosensors (more than the 90 \% of their initial sensitivity after more than 30 days), meets the requirements of the proposed application.

Finally, the fluidic system was applied to the monitoring of the malolactic fermentation of samples recollected during this process for several red wines, showing the results obtained by the proposed system an excellent agreement with those obtained with the standard colorimetric methods.


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Application on environmental monitoring, medical, food and industrial fields.

Magnetic molecularly imprinted polymers. Synthesis and applications
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Molecularly Imprinted Polymers (MIPs) are synthetic biomimetic materials mimicking biological receptors. They are highly cross-linked macromolecular structures towards the template which is then extracted after polymerization, originating cavities (binding sites) complementary to the template molecule, acting as plastic antibodies. \cite{1,2}. The use of magnetic particles (MPs) greatly improves the performance of the biological reaction by increasing the surface area, improving the washing steps and, importantly, minimizing the matrix effect. MPs also allow reduction of reaction times and reagent volumes. In addition, MPs can be easily magneto-actuated using permanent magnets \cite{3}. This work addresses the synthesis of magneto-actuated molecularly imprinted polymers (magnetic-MIPs) in order to merge the outstanding properties of MIPs and MPs. The synthesis comprised the following steps \cite{4}: i) preparation of magnetite nanoparticles (NPs) from co-precipitation of a Fe\textsubscript{2+}/Fe\textsuperscript{3+} solution; ii) reaction of Fe3O4 NPs with tetraethoxy silane, to achieve Fe3O4@SiO2 NPs; iii) reaction with a silanizing agent to achieve Fe3O4@SiO2-MPS NPs; iv) polymerization with a functional monomer, in the presence of the template, a crosslinking monomer, a radical initiator, and a porogenic solvent and, finally, v) extraction of the template to obtain the magnetic-MIPs. In all cases, the selection of the functional monomer was optimized by computational simulation. The non-imprinted polymer (NIP) is synthesized in the same conditions as MIP but without the addition of the template. The characterization of the magnetic-MIP is performed by several techniques as scanning electron microscopy, transmission electron microscopy, XRD, FTIR, VSM and BET analysis. Moreover, the binding capacity towards the specific analyte is demonstrated and compared with the corresponding magnetic-NIP (non-imprinted polymers) taking in account the effect of binding time, buffer and the amount of magnetic-MIP. Finally, magnetic-MIP is used for preconcentration of the analyte from the complex samples allowing its quantification by several magneto-actuating approaches. This novel material was evaluated, among others, in different applications including food pollutants (methyl parathion) and contaminants (histamine) in fish samples as a model and it was also successfully applied for determination of diseases related to hormone (L-thyroxine) giving promising results. This proves the ability of this material to preconcentrate analyte from complex samples and opens the way to incorporate this material in magneto-actuating devices could be used easily in the field of environmental control, food safety, and medical applications.

![Diagram](image)

References
Application on environmental monitoring, medical, food and industrial fields.
The aim of the present study was to investigate the direct electrooxidation of BSA without stabilizer and adsorbent, onto the carbon electrode at various protein concentrations and pH buffer solutions. A simple strategy based on electro oxidation of BSA onto the working electrode of carbon based screen printed electrode, gave a good immobilization support for biological molecules. The study anticipated that this surface not only provide antifouling characteristics, but it can also result into a good conducting transducer interface. Moreover, due to presence of other reactive groups, BSA can provide a soft platform for further surface functionalization. It may also facilitate to understand the proteins interactions with artificial surfaces, selective oxidation within the macromolecular domains and analytical separation.
Label-free detection of aflatoxins B1 and M1 in aptamer assay using spectroscopic ellipsometry

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The method of spectroscopic ellipsometry in total internal reflection (TIRE) configuration was utilised for detection of aflatoxins B1 and M1 in direct assay with specific aptamers. The method of TIRE being a combination of spectroscopic ellipsometry and SPR was proved as a highly sensitive analytical tool particularly suitable for detection of low molecular weight analytes such as mycotoxins [1]. In this work, in addition to traditional TIRE experiments utilising 25nm thick gold films exhibiting SPR, much thinner nano-structured gold films exhibiting LSPR were used. A combination of TIRE and LSPR is a new bio-sensor development capable of "single molecule detection" [2]. The TIRE spectroscopic measurements were performed using the experimental set-up developed on the basis of J.A. Woollam M2000 spectroscopic ellipsometer and described in detail earlier [1]. Nano-structured gold films exhibiting LSPR were prepared by annealing thin (4-10nm) gold layers at 550-580 °C following the procedure described in [3].

Several aptamers sequences specific to aflatoxin B1 and M1 were selected in literature according to their sensitivity and specificity [4,5]. The nucleotide sequences were modified with thiol group in appropriate extremity to allow a one-step covalent grafting on gold at 25 nm and different nominal thickness similar to that described in [6].

Typical results of TIRE detection are the spectra of a phase-sensitive ellipsometric parameter $\Delta$, which are shifted to the shorter wavelength upon binding the analyte molecules to specific aptamer receptors. The "blue" spectral shift is associated with the decrease in the thickness of the aptamer layer due to the aptamer coiling around the target.

The minimal detected concentration of aflatoxins, which depends in the type of aptamer used, is typically of 0.01 ng.ml$^{-1}$ and below. The results of TIRE aptamer assay were compared with those of traditional immunoassay. The use of small size bio-receptors such as aptamers is particularly beneficial for LSPR transducers typically having small evanescent decay length. IgG based antibodies having dimensions comparable with the evanescent decay length yield much higher detection limit around 1ng/ml. The work on development of highly sensitive aptamer based optical biosensors for mycotoxins is currently underway.

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References:
Indirect detection of Microcystins by Acetylcholinesterase-based Amperometric Biosensors

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Microcystins (MC) are a class of hepatotoxins produced by cyanobacteria in surface water. Many authors have reported that MC-LR inhibits the action of intracellular proteins, alkaline phosphatases. Therefore, it has also been shown the stimulation of the acetylcholinesterase enzyme (AChE) activity by the MC-LR. Thus, an AChE-based amperometric biosensor was developed for indirect detection of MC-LR. For construction of the working electrode, a powder graphite paste containing hydroxyethylcellulose (HEC), bovine serum albumin (BSA) and glutaraldehyde (Glu) was prepared. The slurry was incorporated into commercial AChE enzymes extracted from bovine erythrocyte (BE) and electric eel (EE), also commercial butryrylcholinesterase enzyme (BChE) obtained from human serum (HS) as well as genetically modified enzymes derived from \textit{Drosophila melanogaster}. A portion of the sensitive paste was deposited in the screen-printed sensor working electrode and the final biosensors characterized throughout differential pulse voltammetry (DPV) and cyclic voltammetry (CV). Chronoamperometric measurements were performed and the percentage relative enzyme activation (% RA) was then plotted as a function of the MC-LR concentration. Some operating conditions, such as working potential, electrochemical mediator, pH and substrate concentration were optimized. Enzyme activation assays showed that the EE-AChE enzyme showed best results, with % RA > 10 in sublevels contents of MC-LR. The biosensor has shown to be highly precise (CV \approx 8.32\%), sensitive (LOD = 0.27 \mu g .L\textsuperscript{-1} and LOQ = 0.91 \mu g .L\textsuperscript{-1}) and accurate (recovery rates varying from 73 to 105\%). The initial study proved to be the right biosensor to verify the presence of MC-LR in aquatic environments, this being then used in monitoring pollutant seven points of Bacanga River, an important aquatic ecosystem of San Luis, Maranhão State, Brazil. The results indicated that there was no significant contamination by such pollutant in this investigated aquatic ecosystem.

Keywords: Biosensors, Microcystins-LR, Acetylcholinesterase
Abstract

We are demonstrating a new electrochemical microfluidic device as a molecular diagnostics tool; the system facilitates a rapid and inexpensive detection of nucleic acids. The objective is to achieve a low cost, integrated device for use at the point-of-need. The system is low cost (less than 1€ bill of materials) and the time to results is less than 45 min with minimal user intervention, and total integration, truly achieving “sample in-result out”. The power requirement should permit the system to operate with the battery of a mobile device such as a smartphone.

The availability of such a device can be transformational for health care not only in resource-limited environments but also for the rationalisation of health costs in advanced economies. Food safety and quality assurance from farm to fork and the disruptive innovation of HACCP implementation could be another beneficiary of the proposed technological innovation. Finally, environmental monitoring can be improved. The model system used for development is centred on the environmental monitoring field proposing the field detection of toxic algae.

This work has been carried out with the financial support from the Ministry of Economy and Competitiveness, (Seasensing Project, ref.BIO2014-56024-C2-1-R) “Seasensing: Microsystems for rapid, reliable and cost effective detection of toxic microalgae on-site and in real-time”
Determination of hydrochlorothiazide using screen-printed electrodes modified with L-glutamic acid by differential pulse voltammetry

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Hydrochlorothiazide (HCTZ) is a drug widely used around the world for hypertension treatment. The determination of HCTZ in different matrices is currently carried out by means of different analytical techniques, including high performance liquid chromatography (HPLC), which is the analytical technique indicated by the United States Pharmacopeia [1]. However, voltammetric techniques play a fundamental role and are stated as a very notable alternative for the determination of this antihypertensive drug due to their excellent detection and quantification limits, high sensitivity and selectivity and the relatively low cost.

Glutamic acid is one of the 20 most common amino acids, that has a great interest as a modifier since it can be easily immobilized on the electrode surface by both electropolymerization and electrografting approaches.

In this work, both modification approaches have been considered for the development of a glutamic acid modified electrode using a screen-printed carbon electrode as a support. Glutamic acid screen-printed carbon electrodes modified by both approaches electropolymerization (SPCE/PGA) and electrografting (SPCE/EGA) have been compared in terms of their electrochemical characterization and their analytical performance in the determination of hydrochlorothiazide. Moreover, the applicability of SPCE/EGA as a better sensor has been tested through its determination in a commercial antihypertensive drug.

This research develops label-free aptasensors for Codeine (Cod) detection using electrochemical impedance spectroscopy (EIS) technique. EIS is a simple, high-sensitivity, low-cost and rapid transduction principle to follow biosensing events that take place at the surface of an electrode. Besides, the EIS technique is also capable of showing response at very low concentration levels.

Codeine (3-methylmorphine) is an alkaloid separated from opium. This small molecule is extensively used to treat mild to moderate pain and cough suppression in clinics. Despite its medical applications, the abuse of codeine can also create health risks. For this reason, it is very important to find a high sensitive, cheap and rapid method for its detection.

In recent years, a widely variety of conventional methods for the detection of codeine have been developed such as high-performance liquid chromatography, capillary electrophoresis, gas chromatography-mass spectrometry, UV spectrophotometric techniques among others. These methods are expensive and need complex procedures. For this reason, there is a need to develop rapid, cheap and effective devices for detection. To overcome this problem aptasensors is a good alternative.

To achieve the main feature of this work, screen-printed electrodes modified with carboxyl functionalized multi-walled carbon nanotubes (MWCNT-COOH) obtained from Drop Sens (Oviedo, Spain) were used as platforms for impedimetric aptasensing. A covalent immobilization of aminated DNA target using carbodiimide chemistry was performed. In this case, the covalent binding is produced by first electrochemicalgrafting. Electrochemicalgrafting consists of anchoring 4-aminobenzoic acid molecules to the electrode surface through diazonium salt reaction and C-C bond formation. This step leaves benzoic acid moieties exposed to the solution and can be used to immobilize biomolecules that are amino terminated. After that, a blocking step is performed using polyethylene glycol as blocking agent; the label-free assay to detect Cod consists on a simple incubation step with the sample.

To conclude, the developed method could be a useful and promising platform for codeine detection in many applications. In addition, the use of EIS technique does not require any additional and/or labelled species for the transduction. Thus, this detection technique can be used for designing label-free protocols avoiding more expensive and time-consuming assays.
Analytical microsystem for the potentiometric determination of ammonium in space applications

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One of the aims of life support systems in manned space flights of long duration is the water supply for the crew. Water is recycled from urine, cabin condensate and grey water [1]. To verify that the resulting water provided by the regeneration process meets the requirements established by the European Space Agency (ESA), automatic on-line miniaturized analyzers with high robustness, low cost and weight, and with low sample and reagent consumption are needed.

In this work, the development of a polymer-based continuous flow potentiometric microanalyzer to monitor ammonium in water samples is presented. The analytical microsystem, fabricated using Cyclic Olefin Copolymer (COC) and smaller in size than a credit card, integrated two ammonium selective electrodes (one as reference electrode and the other one as indicator electrode) and a gas-diffusion module. The ion selective electrodes were fabricated using epoxy-graphite as conductive support and an ammonium PVC membrane. The gas-diffusion module was based on a polyvinylidene fluoride (PVDF) membrane.

The proper operation under microgravity conditions was tested using a compact and autonomous experimental setup which minimized the reagents and energy consumption.

Analytical features obtained during the characterization of the microanalyzer met the requirements needed for real space applications.

References:

The study of DNA and its relationships with potentially toxic compounds has been the subject of many publications in recent decades. Glyphosate-based herbicides have been widely used in the world, mainly with the development of genetically modified crops resistant to it. Considered initially a non-toxic pesticide, recent studies have shown the decreased activity of some enzymes in laboratory animals exposed to them. The present work evaluated the behavior of Calf Thymus DNA in the absence and presence of glyphosate, using spectrophotometric methods, in order to estimate possible structural interactions. Spectrophotometric characterization studies, pH variation studies (7.2 and 12), as well as the time of exposure of the DNA to glyphosate (10, 30 and 60 min) were carried out. The study evidenced effects of hyperchromism and hypochromism on the DNA signal when the herbicide was present in the analytical medium. It has been found that such effects depend on the concentration of the pesticide under study, and the behavior may vary in relation to the change in pH from denaturation of the genetic material. It is estimated that the data found may show different forms of interaction between glyphosate and the helical antiparallel structure of DNA, such interactions being more damaging to DNA when at pH 7.2.

**Key words:** DNA Calf Thymus, glyphosate, interaction studies.
For all living aerobic organisms, the molecular oxygen (O\textsubscript{2}) is required for the cellular respiration which is reduced to water with the inevitable formation of Reactive Oxygen Species (ROS). The recent growth in the knowledge of ROS demonstrates that a balance between ROS and antioxidants is necessary to maintain the normal physiological function. Nevertheless, if the ROS overwhelm the body's ability to regulate them, a condition known as oxidative stress ensues, involving in oxidative injury in cellular components and consequently the development of a number of diseases. Antioxidants are well-known for their ability to scavenge ROS and this provided protection stimulates search by natural antioxidants that can prevent or minimize the oxidative damage. In this view, the Brazilian flora presents a vast biodiversity with great chemical and pharmacological potentialities unexplored.

The main objective of this work is to identify new potential natural sources of antioxidants. For this, the antioxidant capacity based on the superoxide radical (O\textsubscript{2}•\textsuperscript{-}) scavenging ability was determined by a spectrophotometric bio-assay and an amperometric biosensor. In both cases, the superoxide radicals were generated in vitro during the catalytic oxidation of hypoxanthine by Xanthine OxiDase (XOD). The miniaturized bio-assay allows a rapid and reasonably accurate measurement of fruit extract. The design of the biosensor is based on the measurement of antioxidant oxidation at E=0V vs. Ag/AgCl with PEDOT modified screen printed electrode. Hypoxanthine addition triggers the O\textsubscript{2}•\textsuperscript{-} production which is scavenged by the antioxidant, inducing the decrease of the measured current due to the antioxidant concentration reduction. Thus, this decrease can be correlated to the antioxidant capacity.

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MOLECULARLY IMPRINTED POLYMERS FOR BIOTIN AND BIOTINYLATED MOLECULES. A PROMISING MATERIAL FOR RAPID DIAGNOSTIC TESTS

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Keywords: MIPs, magnetic MIPs, biotin, lateral flow, biosensors

Molecularly Imprinted Polymers (MIPs) are synthetic biomimetic materials mimicking biological receptors. They are highly cross-linked macromolecular structures towards the template which is then extracted after polymerization, originating cavities complementary to the template molecule.¹ Due to the wide range of applications, the synthesis of streptavidin-mimicking molecularly imprinted polymers and their downstream applications are presented. On one hand, the synthesis and characterization of molecularly imprinted polymers towards biotin², as well as the separation of biotinylated biomolecules and the detection of their targets, are presented. On the other hand, the integration of the biotin-MIP in a lateral flow strip is also described for the first time, for the detection of double-tagged PCR amplicon.³ The application of this NALF (nucleic acid lateral flow) device based on MIPs is demonstrated for communicable and non-communicable diseases, taking as a model the detection of the waterborne pathogen *E. coli* and the quantification of circulating tumor cells of breast cancer, respectively, demonstrating that this device is extremely versatile for an enormous range of analytes.

![Figure 1](image_url)

*Figure 1. SEM images with 25.00 and 4.00 K X magnification of A) MIP and B) MIP deposited on the nitrocellulose membrane on the Lateral Flow set-up.*
Figure 2. Results obtained for different amounts of double-tagged amplicon range from (0-140 ng·mL⁻¹); for MIP in a lateral flow approach.

References

**Low cost platform for detection of cardiomyopathy causing mutations using EPEX reaction**

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The detection of single nucleotide polymorphisms (SNPs) is of great importance in life sciences, having application in personalised medicine, patient stratification, forensics, non-invasive prenatal screening as well as providing information regarding predisposition to disease. SNPs can be detected in an array format using the array based primer extension, and we have exploited this technique to develop electrochemical primer extension reaction (EPEX). In electrochemical primer extension, probes are immobilised on electrode surfaces, and are designed to hybridise to a single stranded PCR amplicon just one base lower than the location of the SNP. Redox labelled ddNTPs are then introduced, with each of the four ddNTPs (G, A, T and C) having a different redox label that can be used to identify the SNP. In the work reported here we developed cost-effective platform for detecting SNPs at identified locations, using electrochemical detection of ddNTPs labelled polyoxometalates (Keggin and Dawson). Polyoxometalates (POMs) are nanometer sized transition metal oxide clusters that have a spectrum of potential applications in catalysis, material science, and medicine. Gold electrodes were functionalised with probes designed to bind to an amplicon of the MYH7 gene. Asymmetric PCR was used to produce single stranded DNA containing 4 different SNP(s) to be interrogated, which subsequently hybridised to the specifically designed and immobilised probes. Following hybridisation, POM-labelled ddNTPs were introduced and the immobilised probes were elongated by one base complementary to the SNP. Following elongation, the redox label of the incorporated ddNTP was measured using differential pulse voltammetry (DPV). Responses obtained from Dawson and Keggin ddNTPs were compared and applied to multiplexed analysis of a PCR amplicon.
Volatile phenols such as 4-ethylguaiacol, 4-ethylphenol and 4-ethylcatechol, among others, are natural constituents of wine, which play a role in its aroma. But if their concentration levels are high, volatile phenols can be a defect, deteriorating wine quality. In red wines they act, when at low concentrations, as a distinctive aged character; at the higher concentrations they can produce undesired aromas and flavors, characterized by the oenologist as “phenolic”, “leather” or “barnyard” character.

There are various ways to quantify volatile phenolic compounds. This communication describes an electrochemical biosensor based on a graphene platform using the laccase enzyme, which can be an alternative in front of spectrophotometric reactions or heavy instruments as HPLC or HPLC-MS. Biosensor-based methods provide some advantages such as easy and fast operation, low maintenance costs and high sensitivity.

Thus, the goal of this study is to develop biosensors for the determination of phenols, via cyclic voltammetry (CV), using laccase enzyme on platforms of electroreduced graphene (ERGO) as key nanotechnology material. In this case, Laccase is covalently immobilized to graphene via carbodiimide chemistry. Full characterization is given, together with the final application in the wine field.

References:
Potentiometric microanalyzer with an enhanced sensitivity for the determination of chloride ion

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Cystic fibrosis is a disease caused by a mutation in a gene characterized by an excessive concentration of salts in sweat, increasing the viscosity of the mucous membranes and producing obstructions, especially in lungs.

In order to diagnose this disease, it is necessary to have a clinical suspicion and get positive results in a chloride analysis test in sweat. A concentration higher than 60 mmol L⁻¹ Cl⁻ is considered pathological, being considered as normal when it is lower than 30 mmol L⁻¹.

For this reason, it is necessary analytical instrumentation with high sensitivity besides of portability, low reagents consumption, high level of automatization and short analyses time, among others features.

In this work, the development of a continuous flow microanalyzer to monitor chloride ion in sweat using differential potentiometric measurements is presented. The modular microsystem integrates two chloride selective electrodes (one as reference electrode and the other one as indicator electrode), made of a Ag/AgCl screen-printed paste.

The results obtained after the optimization process show a linear working range which contains the range of physiological concentrations, with an excellent repeatability and with an increased sensitivity of 78 % compared to a conventional potentiometric calibration.