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Book of Abstracts

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ABSTRACT BOOK

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SPONSORS AND EXHIBITORS

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GENERAL PROGRAM

GENERAL PROGRAM

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PL-2	Analytical micro and nanotechnologies for modern electroanalysis: present and horizons *Jesús Alberto Escarpa Miguel*
PL-3	Liquid chromatography – mass spectrometry: challenges and applications *Maria do Rosário G.R.M. Domingues*
PL-4	From environmental sciences to quality control: towards multidimensional analytical chemistry *Armando C. Duarte*

PL-1

ANALYTICAL CHEMICAL LUMINESCENCE TODAY AND TOMORROW

Aldo Roda

Department of Chemistry "G. Ciamician",

Alma Mater Studiorum-University of Bologna, Italy

Chemical luminescence, which includes all the techniques where cold light is produced by a chemical reaction, is a well-established detection principle with a wide range of applications from the life sciences to environmental, food, and biomedical analysis [1]. The main features are the high detectability, rapidity, and suitability for miniaturization in biosensor format. The most successful are bio-chemiluminescence (BL-CL) and electrogenerated chemiluminescence (ECL) labels for bioassay and almost all automated immunoanalyzers in clinical chemistry laboratory are based on these principles. Nowadays the possibility to detect and quantify light down to a few photons by use photomultiplier tubes and new generation of photodiodes, or imaging systems like CCD or CMOS, make BL and CL unique tools for development of ultrasensitive analytical methods combined with a simple instrumentation. Several new concept assays and devices have been reported to integrate BL/CL-based detection in miniaturized multiplexed systems for point-of-care applications, lab-on-chip, whole-cell biosensors, and lab-on-paper based devices. Besides, taking advantage of enhanced performance of smartphone phone integrated camera, BL-CL biosensors have been developed with the potential to enter in ordinary life for home self-diagnostics. [2]. The ongoing trend in the development of CL assays relying on the use of nanostructured materials as sensors or new high efficient CL nanocatalysts to improve the light efficiency which is still the main limitation. New tools for investigating molecular targets at the cellular level are now available thanks to the obtainment of BL luciferases with improved spectral properties (e.g. with higher emission efficiency and different emission wavelengths). This enabled not only the development of highly sensitive cell-based multiplexed assays based on reporter gene technology, but also to conceive new assays for monitoring the very first events of signal transduction, i.e., via protein-protein interactions, based on new generation hybrid bioluminescence resonance energy transfer (BRET) using quantum dots (QDs) as the acceptor and split complementation assays. New BL models for *in vivo* BL imaging with higher tissue penetration and signal quantification thanks to BL proteins emitting in the far red or near infrared will be helpful in exploring the complex signaling networks useful for cancer researches. The mechanism of new BL organisms in the deep ocean and in land such as glow mushroom will be expanded the analytical tools. Thermochemiluminescence (TCL) is an emerging technique that will improve the analytical performance of these methods thanks to the possibility to detect the label without the addition of chemicals being the light generated by a simple thermal decomposition of the emitters i.e. a reagent free system. An overview of BL and CL ongoing research related will be provided to highlight current trends, technical challenges and foresee future directions.

[1] Roda, A.; Mirasoli, M.; Michelini, E.; Di Fusco, M.; Zangheri, M.; Cevenini, L.; Roda, B.; Simoni, P. *Biosens.Bioelectron*. **2016**, 76, 164-79.

[2] Roda, A.; Michelini, E.; Zangheri, M.; Di Fusco, M.; Calabria, D.; Simoni, P. *TrAC,* doi: 10.1016/j.trac.2015.10.019

PL-2

ANALYTICAL MICRO AND NANOTECHNOLOGIES FOR MODERN ELECTROANALYSIS: PRESENT AND HORIZONS

Alberto Escarpa
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Analytical microfluidics and nanotechnologies are the paradigm of the nowadays miniaturization concept in analytical chemistry.

The main key advantage of microfluidic chips (MC) is the ability to perform analysis in real time in very short time and with extremely low sample volumes. Electrochemical detection coupled to MC is a valuable detection principle that provides inherent sensitivity, permits miniaturization, and is highly compatible with micro- and nanotechnologies.

On the other hand, as an important block in nanotechnology, nanomaterials (NMs) are very unique structures, not only because their inherent high surface area, but also because of their tunable electronic properties and controlled functionality. Relevant examples include carbon nanotubes, graphene and metallic nanowires.

The use of NMs as electrochemical detectors in MC is a convenient marriage for improving the overall analytical performance, providing high currents –associated with the large surface areas- thereby enabling large-scale redox conversion. Such remarkable features increased the analytical selectivity and sensitivity as well as the resistance to passivation, yielding thus very good reproducibility.

Because of the extremely low sample volumes introduced into MCs, sensitivity is often low and represents the main drawback of these systems. A solution to this problem lies on the on the exploitation of the surface characteristics of NMs, leading thus to a novel generation of MC.

In this communication, I will discuss the analytical potency of NMs for electrochemical sensing on MCs and exciting horizons in the field towards novel sensing protocols.

[1] Crevillén, A. G.; Ávila, M.; Pumera, M.; González, M. C.; Escarpa, A., *Anal. Chem.* **2007**, 79, 7408.
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[5] García, M.; Alonso-Fernández, J. R.; Escarpa, A. *Anal. Chem.* **2013**, 85, 9116
[6] Batalla, P.; Martín, A.; López, M. A.; González, M. C.; Escarpa, A. *Anal. Chem.* **2015**, 87, 5074.

PL-3

LIQUID CHROMATOGRAPHY - MASS SPECTROMETRY: CHALLENGES AND APPLICATIONS

Rosário Domingues

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Liquid chromatography-mass spectrometry (LC-MS) has become increasingly popular and is nowadays a key technique in different fields of research, industry and clinical laboratories. It is being routinely used for quantitative and qualitative analysis of specific molecules, such as pesticides, foods components or contaminants, pharmaceutical drugs, substances used in doping, among others. LC-MS is also a crucial analytical tool in the modern Omics advanced platforms metabolomics, lipidomics and proteomics.
The applicability of LC–MS analysis has expanded with the advances of mass spectrometry technologies, namely the development of different types of ions sources (electropray, nanoelectrospray and atmospheric pressure ionization), new mass analysers (linear ion trap, orbitrap, others) and mass spectrometry platforms (Q-TOF, TOF-TOF, iontrap-orbitrap) with increased resolution and sensitivity. Additionally, the LC-MS give more complete, selective and specific information than the tradition approaches such as LC—UV, because it allows the confirmation of the identity of all the compounds, from the information gathered by the MS analysis, namely the identification of molecular ions in MS and identification of specific fragmentation patterns in the MS^n tandem mass spectra. It also allows the study of complex samples, and can be selectively used to perform either target or untargeted analysis, depending on the LC-MS platforms and experimental conditions.
The versatility of LC-MS platforms admits a wide range of applications. However for each application, the selection of the appropriate LC-MS interface is important for success of LC-MS experiments Specific knowledge on mass spectrometry data analysis and interpretation is essential for the validation of results and development of new applications.
In this presentation we will discus the key steps of LC–MS platforms, emphasizing the operation modes and practical aspects of the different MS instruments, showing specific examples of application and addressing specific challenges and futures applications.

Acknowledgements:
Thanks are due to University of Aveiro, FCT/MEC, European Union, QREN, COMPETE for the financial support to the QOPNA research Unit (FCT UID/QUI/00062/2013), through national founds and where applicable co-financed by the FEDER, within the PT2020 Partnership Agreement, and also to the Portuguese Mass Spectrometry Network (REDE/1504/REM/2005).

PL-4

FROM ENVIRONMENTAL SCIENCES TO QUALITY CONTROL: TOWARDS MULTIDIMENSIONAL ANALYTICAL CHEMISTRY

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In the second half of last century, Analytical Chemistry developed thanks to scientific interests associated with the needs for detecting and solving environmental quality issues. The production of large amounts of data by analytical methodologies with lack of reproducibility led to a further stage of development, which is the incorporation of quality control and quality assurance in the analytical procedures. By the end of last century Analytical Chemistry became very much associated with metrological issues which had to be applied and specifically developed to the laboratory in order to obtaining experimental data to support decisions on food safety, health and environmental protection, and sustainable development. Since Analytical Chemistry, among others, was an important topic at the University of Aveiro, some examples can be drawn from local academic in order to show how this general view was translated into research and training activities associated with both environmental sciences and quality control. Ria de Aveiro, a local lagoon close to the University of Aveiro, has several environmental quality problems and it has served as a natural laboratory for developing research and applying Analytical Chemistry with a view fit for the above mentioned purposes. Furthermore, in terms of training, a pioneering thematic in Analytical Quality Control was incorporated in courses of Chemistry and Environmental Sciences which had a large impact on the following generations of students.

More recently, the interfacing of multidimensional chromatography (such as two dimensional (2D) comprehensive liquid chromatography, LC×LC) coupled with either online or offline multidimensional analytical instrumentation (such as high resolution mass spectrometry, 2D nuclear magnetic resonance (NMR) spectroscopy, excitation-emission matrix (EEM) fluorescence spectroscopy) has provided a plethora of large data sets containing chemical information that need to be handled properly in order to become manageable and useful. When such N-dimensional analytical strategies are applied to characterize the natural organic matter (NOM) of complex samples such as atmospheric aerosols, the task of handling and re-arranging such data sets may become an endeavour of utmost importance for decoding the structural features of NOM present in fine atmospheric aerosols. Apart from the intricacies of data acquisition from different instrumental setups available, there are also the difficulties of multivariate analysis of complex data that need to be translated into knowledge about the huge variety and inherent complexity of the molecular structures and formation mechanisms of the Organic Aerosol (OA) fraction.

The application of multivariate and computer based concepts to the interpretation of chemical data obtained from N-dimensional analytical methods can provide insights mainly when dealing with complex samples and “messy data”. This lecture highlights the specific problems when dealing with N-dimensional experimental designs, and incorporation of quality control strategies at the acquisition stage of N-dimensional analytical data, pre-processing options fit for multivariate analyses, and definitions of sound principles for choosing fit for purpose models in order to avoid misinterpretations of data. Furthermore, this work attempts to follow the application of the above mentioned principles with data from LC×LC and a combination of excitation-emission matrix (EEM) fluorescence spectroscopy and 2D correlation of FTIR and NMR spectroscopies, applied to the characterization of NOM from atmospheric aerosols.

KEYNOTE LECTURES

K-1	Vibrational spectroscopy: applications in forensic and medicinal chemistry *Maria Paula Marques, A.L.M. Batista de Carvalho, D. Gonçalves, S.F. Parker, G. Cinque, L.A.E. Batista de Carvalho*
K-2	Point of care testing of nitrites: construction of enzyme biosensors for environmental, food, and biomedical applications *Maria Gabriela Almeida*
K-3	Atomic spectrometry in clinical chemistry/toxicology and forensic sciences – overview and highlights *Agostinho Almeida*
K-4	Nuclear analytical techniques for speciation of trace elements in environmental sciences *Susana Marta Almeida, M. Almeida-Silva, N. Canha, A.V. Silva, J. Lage, C. Galinha, C. Ramos1, T. Faria, I. Dionísio*

K-1

VIBRATIONAL SPECTROSCOPY APPLICATIONS IN FORENSIC AND MEDICINAL CHEMISTRY

M.P.M. Marques[1,2], A.L.M. Batista de Carvalho[1], P.S.C. Medeiros[1], D. Gonçalves[3-5], S.F. Parker[6], G. Cinque[7], D. Gianolio[7] and L.A.E. Batista de Carvalho[1]

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[3]Research Centre for Anthropology and Health (CIAS) and [4]Laboratory of Forensic Anthropology, Centre of Functional Ecology – Univ. Coimbra, PT

[5]Archaeosciences Lab, Directorate Gen. Cultural Heritage and LARC/CIBIO/InBIO,PT

[6]ISIS Facility, STFC Rutherford Appleton Lab, Chilton, Oxfordshire, UK

[7]Diamond Light Source, Harwell Science and Innovation Campus, Chilton, Oxfordshire, UK

In the last decades, vibrational spectroscopy has arisen as a suitable and extremely accurate analytical tool for the characterisation of biological systems. Its high sensitivity, non-invasive nature and real-time molecular imaging capability, without the need for dyes or external probes, allows to attain extremely accurate biochemical information and images of cells and tissues, in both *in vitro* and *in vivo*/*in situ* settings. In particular, Raman confocal microspectroscopy of cells constitutes a unique probing technique, yielding precise information on functionality and metabolic profile under distinct conditions, namely as a response to chemotherapeutic agents [1]. Additionally, coupling the complementary technique Fourier transform infrared (FTIR) microspectroscopy to the brilliance of synchrotron radiation sources leads to a massive increase in the quality of the results, giving rise to spectra with subcellular resolution and unsurpassed S/N ratio. This is an unmatched approach for routine chemical profiling of cells, allowing to examine cellular environments and monitor drug´s bioavailability, biodistribution and target site accumulation after administration, as well as drug delivery across biological barriers and cellular response to treatment [2,3].

Bone is a biphasic material comprising an organic component (mostly collagen) woven into an inorganic matrix of bioapatite ($Ca_5(PO_4)_3OH$). Skeletal human remains are often found in archaeological and forensic contexts, and their accurate analysis aiming at the study of past populations or at victim identification is of unquestionable relevance. Vibrational spectroscopy (*e.g.* infrared [4], Raman [5] and Inelastic Neutron Scattering (INS) [6]) have been shown to be extremely suitable for probing bone diagenesis (*post-mortem* processes prompting physical/chemical alterations), providing reliable clues for a precise sample characterisation (*e.g.* chemical composition, moisture, temperature). Particularly for human bones subjected to burning events, bioanthropologists are faced with problems when establishing individual biological profiles, as burning leads to considerable dimensional variations due to heat-induced modifications in the bone´s crystal structure [7] therefore interfering with the reliability of the available meth. conceived for unmodified dimensions.

Acknowledgements: Portuguese FCT - UID/MULTI/00070/2013, PTDC/QEQ-MED/1890/2014. ISIS and DLS (UK), for access to neutron beam facilities and B22/MIRIAM (European programme Calypso FP7).

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K-2

POINT OF CARE TESTING OF NITRITES: CONSTRUCTION OF ENZYME BIOSENSORS FOR ENVIRONMENTAL, FOOD AND BIOMEDICAL APPLICATIONS

Maria Gabriela Almeida[1,2]

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Point of care testing (POCT) of nitrites (NO_2^-) represents an important R&D field, targeting key applications in the environmental, food, clinical and biomedical markets. In fact, the extensive use of nitrite precursors as fertilizers is of great concern in the balance of ecosystems and to the contamination of groundwater supplies. Because the continuous ingestion of nitrites brings potential hazards for human health, the European Union implemented a number of directives that restrict the level of these ions in drinking waters [98/83/EC] and food products [2006/52/EC]. On the other hand, the detection of nitrites in physiological fluids is commonly used in clinical diagnosis [1]. Noteworthy, nitrites monitoring has been gaining an increasing relevance in biomedical research due to the recognition of its important role as a mediator of blood flow regulation, cell signaling, energetics and tissue responses to hypoxia [2]. Unfortunately, none of the classical protocols for nitrites assessment meet all requisites demanded of an analytical assay such as simplicity, sensitivity, selectivity, low detection limit and quickness. Therefore, novel POCT enabling immediate but effective readings of NO_2^- in complex matrices are urgently needed.

In the last years, our group has been focused on the development of enzyme-based biosensors for nitrites measurement. Several approaches using a bacterial nitrite reductase (NrfA) were successfully implemented using electrochemical transducers in both 2nd and 3rd generation formats. Typically, the biosensors display a high selectivity and sensitivity that come from the enzyme ability to discriminate the analyte against interfering species and catalyse the NO_2^- reduction with high turnover rates, respectively [3-6]. A major progress was recently made with disposable, cheap and easy-to-use screen-printed electrodes modified with a carbon ink/NrfA composite, which were effectively applied in the analysis of milk, drinking water and physiological samples. To remove oxygen interference, the bi-enzyme system *glucose oxidase/catalase* was successfully used, thus eliminating the need of sample degassing and moving nitrite diagnostics from the bench to the field [7].

Acknowledgements: This work was supported by Unidade de Ciências Biomoleculares Aplicadas-UCIBIO, which is financed by national funds from FCT/MEC (UID/Multi/04378/2013) and co-financed by the ERDF under the PT2020 Partnership Agreement (POCI-01-0145-FEDER-007728).

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K-3

ATOMIC SPECTROMETRY IN CLINICAL CHEMISTRY/TOXICOLOGY AND FORENSIC SCIENCES – OVERVIEW AND HIGHLIGHTS

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Classical atomic spectroscopy, especially flame (atomic emission) photometry (FP), flame atomic absorption spectrometry (FAAS) and graphite furnace atomic absorption spectrometry (GFAAS), are outstanding landmarks in clinical chemistry/ toxicology because of their noteworthy role in understanding the effects of metals and metalloids [macro minerals (Na, K, Ca, Mg) and trace elements (e.g., Fe, Cu, Zn, Se, Mn, Al, Pb, Cd, As, Cr…)] in human health and disease [1,2][1].

In the last two decades, many clinical laboratories moved from these classical techniques toward inductively coupled plasma-mass spectrometry (ICP-MS), an elemental mass spectrometry technique, since it constitutes an analytical tool even more sensitive than GFAAS. Furthermore, it offers multi-element analysis capability (with a wide elemental coverage, including most non-metals) with a high sample throughput (several tens of elements can be measured in few minutes), which makes it very attractive for both routine and research purposes. Additionally, ICP-MS has a unique feature, with great importance in clinical chemistry/toxicology and forensic sciences: the possibility of measurement of stable isotopes [3, 4].

In this communication we will present a historical overview of atomic spectroscopy development, illustrated with examples of its importance for the clinical and forensic laboratories. The focus will be placed on ICP-MS, our recent work and some particular research topics, such as, firing distance estimation, postmortem interval estimation, differential diagnosis of death by drowning, elemental mapping of trace elements levels in brain tissue (besides some food and environmental applications).

Finally, the potentialities of the novel *single particle*-ICP-MS methodologies will be highlighted. High-throughput methodologies for the simultaneous measurement of nanoparticles (NP) size and concentration are now available. And the “particle” may be a “cell”, which means that the individual analysis of isolated cells is now possible.

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[1] FP (first commercial equipment was launched in 1949) “paved the way for modern clinical laboratory medicine with the concept of looking at the biochemistry of a patient [serum Na and K levels, in the case] almost in real-time” [1]. FAAS (introduced in the 1960’s), enabled the fast, easy and accurate determination of Ca, Mg, Zn and Cu in serum. And, with the utilization of analyte preconcentration procedures, also the determination of Pb in whole blood, for example. GFAAS (introduced in the 1970’s), because of its 100-1000 x better detection capability (compared to FAAS), made possible the determination of almost all metals and metalloids at ppb levels. One single example is sufficient to demonstrate its tremendous contribute for clinical chemistry/toxicology: the elucidation of the so-called “dialysis dementia”, the role of aluminum and its monitoring in patients undergoing chronic dialysis therapy.

K-4

NUCLEAR ANALYTICAL TECHNIQUES FOR SPECIATION OF TRACE ELEMENTS IN ENVIRONMENTAL SCIENCES

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Acute and chronic exposures to Airborne Particulate Matter (APM) have been linked, in epidemiological studies, to increased mortality and to a wide spectrum of respiratory and cardiovascular disorders. Several parameters can influence the toxicity of the APM that is present in the outdoor, indoor or workplaces: the particle size distribution, the bulk chemical composition and the contents of trace element, acid and sulphate. However, neither the responsible component, nor the mechanism of adverse health effects is clearly known. Therefore, an accurate knowledge on the nature of the APM and, above all, on the identification of their major sources and pathways through the environment is needed to identify mitigation actions. These focused mitigation actions are essential to enforce an effective reduction of the APM and a decrease of the associated adverse health effects.

The ability of the Nuclear Analytical Techniques (NATs) to analyze solid phase samples, without the need for sample dissolution or digestion and with a high degree of sensitivity and selectivity makes them particularly suitable for the elemental analysis of APM filters. Their multi-elemental detection capability is essential in fingerprinting and apportionment of sources, because each source is characterized by a given set of elements.

The main goal of this work is to present the achievements obtained within more than 15 years of activities related with the use of NATs on the analysis of APM sampled in outdoor, indoor and industrial environments. The results presented confirm the relevance of NATs as efficient analytical techniques not only in the characterization of APM, but also in source apportionment, identification of long range transport and health assessment studies.

Acknowledgements: The authors gratefully acknowledge Fundação para a Ciência e Tecnologia (FCT) for funding S.M. Almeida contract (IF/01078/2013) and the UID/Multi/04349/2013 project.

INVITED COMMUNICATIONS

IC-1	Self-assembled nanostructured electrodes for enzyme based biosensors and antibody/antigen interactions *Luís C. Almeida, Telmo O. Paiva, Inês Almeida, Joaquim T. Marquês, Ana S. Viana*
IC-2	Platinum microelectrode arrays for high spatio-temporal resolution recordings in the brain: surface properties, electrochemical characteristics and *in vivo* analytical performance *Ana Ledo, C.F. Lourenço, J. Laranjinha, G.A. Gerhardt, R.M. Barbosa*
IC-3	Electroanalytical detection of telomeric G-quadruplexes and their application in nanomedicine cancer treatment *Ana Maria Chiorcea-Paquim, A.D.R. Pontinha, A. M. Oliveira-Brett*
IC-4	Application of analytical tools to molecular toxicology *Alexandra M.M. Antunes*
IC-5	Analytical chemistry supporting pharma industry *Andreia Correia, Iva Costa, Katalin Kováts, Sónia Amaral, Vera Fernandes*
IC-6	Environmental proteomics: approaches and limitations *Mário S. Diniz*
IC-7	Pb isotopes: a tool in biogeochemical studies *Miguel Caetano, Pedro Brito, Rute Cesário, Nuno Fonseca, Bárbara Anes, Carlos Vale*
IC-8	New nanostructured electrochemical biosensors for analysis of beverages *Madalina M. Barsan, Christopher M.A. Brett*
IC-9	New wet and dry analytical methodologies for coffee analysis *João R. Santos, João A. Lopes, António O.S.S. Rangel*
IC-10	Assessment of human neutrophils' viability by multiple analytical approches *Marisa Freitas, Tânia Soares, Carina Proença, Daniela Ribeiro, Eduarda Fernandes*

IC-1

SELF-ASSEMBLED NANOSTRUCTURED ELECTRODES FOR ENZYME-BASED BIOSENSORS AND ANTIBODY/ANTIGEN INTERACTIONS

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The immobilization of biomolecules on surfaces is a crucial step in the development of electrochemical or optical biosensors. We have been exploring simple methods to chemically modify gold substrates with the goal of creating robust assemblies of biologically active enzymes [1,2] or antibodies [3,4].

In this context, two main approaches to biofunctionalize carbon or gold electrodes will be discussed. First, the spontaneous formation of polydopamine (PDA) films on graphitic surfaces is presented. PDA is a biomimetic and highly functional material enriched with quinone groups, which are able to covalently bind target biomolecules, such as Laccase and Glucose Oxidase. The enzyme modified electrodes with biocompatible PDA films display high catalytic activities and are promising for applications in biosensors or as electrode materials for biofuel cells.

The second methodology discussed consists on the one-step reaction between amine groups of biomolecules and carbon disulfide, in the presence of gold surfaces. The dithiocarbamate group thus formed is strongly linked to the gold surface, through a bidentate N-C-S2 resonance structure. This method, if carried out in the presence of metallic or metal oxide nanoparticles, can be used to prepare nanostructured biosensing interfaces, and is an attractive alternative to the common multi-step alkanedithiol monolayers formation. The potential of these novel interfaces is demonstrated in the development of universal, non-labelled optical immunosensors, suitable for the specific and sensitive antibody/antigen detection.

The combined information provided from several surface sensitive techniques, namely electrochemical methods, ellipsometry, surface plasmon resonance, and atomic force microscopy is determinant to correlate between morphology, thickness, biomolecule surface density and the analytical performance of the modified surfaces prepared.

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IC-2

PLATINUM MICROELECTRODE ARRAYS FOR HIGH SAPTIO-TEMPORAL RESOLUTION RECORDINGS IN THE BRAIN: SURFACE PROPERTIES, ELECTROCHEMICAL CHARACTERISTICS AND *IN VIVO* ANALYTICAL PERFORMANCE

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Brain function integrity depends on the tight coupling between glutamatergic neurotransmission and energy metabolism. During neuronal activation, local metabolic rate may increase as much as 50% from basal values and consequently changes in blood flow and nutrient supply must be strictly attuned with neuronal activity. Modern concepts of neurometabolism propose that energy metabolism in compartmentalized in the brain, with neurons operating mainly on oxidative metabolism, while astrocytes depend heavily on glycolytic energy production. Our understanding of such mechanisms depends on the availability of adequate analytical tools to monitor these metabolic markers *in situ* and *in vivo*.

Micro-biosensors coupled with fast electrochemical techniques are very attractive for monitoring the concentration dynamics of both electroactive (oxygen) and non-electroactive (e.g. glutamate, glucose and lactate) neurotransmitters and metabolic substrates in the brain with high spatial and temporal resolution and minimal tissue damage. Measuring these non-electroactive compounds requires biosensors based on immobilized oxidase enzymes that produce the reporter molecule H_2O_2.

Multi-site platinum microelectrode arrays (MEAs) with a well-defined and highly reproducible geometrical configuration allow multi-analyte detection in different brain areas. Platinum has excellent catalytic properties for the oxidation of H_2O_2 and is very biocompatible allowing for long-term implantation.

Here we show the morphological and electrochemical characterization of Pt-based MEAs using scanning electron microscopy, cyclic voltammetry and electrochemical impedance spectroscopy. We have characterized the oxidation and reduction reactions of H_2O_2 and O_2 on the Pt surface. Additionally, we have developed oxidase-modified MEAs for *in vivo* measurements of glucose and lactate. Finally, we show the application of MEA based (bio)sensors for *in vivo* recording of pO_2, glucose and lactate in the brain of anesthetized and freely moving rats. High-frequency acquisition of data and offline signal processing provides useful information regarding local-field potential related currents, which can be correlated with animal behavior. Our data highlights the unique suitability of these Pt-based MEAs both for direct recording of O2 as well as the development of enzyme-based microbiosensors for non-electroactive neurochemicals.

Acknowledgements: This work was funded by COMPETE and FCT under the strategic project UID/NEU/04539/2013

IC-3

ELECTROANALYTICAL DETECTION OF TELOMERIC G-QUADRUPLEXES AND THEIR APPLICATION IN NANOMEDICINE CANCER TREATMENT

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Telomeres are responsible for the protection of the ends of linear chromosomes, being involved in greater than 80% of all cancers. The formation of G-quadruplexes (GQs) at the telomere level has been shown to disrupt the telomere capping and maintenance, leading to tumor cell death by apoptosis, which revealed the crucial role of these structures as targets for anticancer drugs. Among GQ ligands, the acridines have been used as chemotherapeutic agents in human cancer. More recently, a new series of triazole-linked acridine ligands, e.g. GL15, with enhanced selectivity for human telomeric GQs binding versus duplex DNA binding have been designed, synthetized and evaluated [1].

The *Tetrahymena* telomeric repeat sequence d(TG_4T) forms parallel-stranded tetra-molecular GQs in the presence of Na^+ and K^+ ions and is considered to be a simple model to study the telomeric DNA interactions with drugs. The d(TG_4T) interaction with the triazole-acridine conjugate GL15 was investigated at the single-molecule level, using the combination of two powerful analytical techniques, atomic force microscopy (AFM) and voltammetry [2].

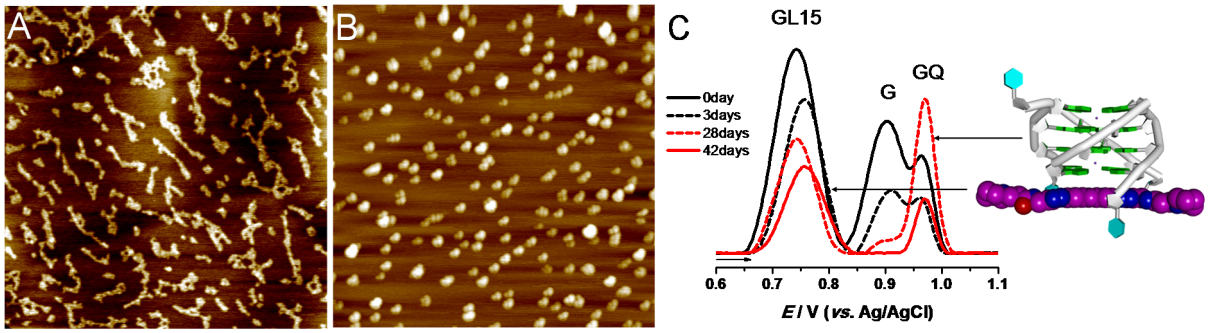

***Figure 1*:** GL15–d(TG4T) after different incubation times in the presence of K+ ions: (A, B) AFM images and (C) differential pulse voltammograms baseline corrected.

The GL15 interacted with d(TG_4T) in a time dependent manner and GQ formation was detected. AFM showed the adsorption of GQs as small d(TG_4T) spherical aggregates, and voltammetry showed the decrease and disappearance of the GL15 and the guanine oxidation peak currents, and the appearance of the GQ oxidation peak (Fig. 1). The GL15 molecule strongly stabilized and accelerated the GQ formation in both Na^+ and K^+ ion-containing solution, although only K^+ promoted the formation of perfectly aligned tetra-molecular GQs.

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IC-4

APPLICATION OF ANALYTICAL TOOLS TO MOLECULAR TOXICOLOGY

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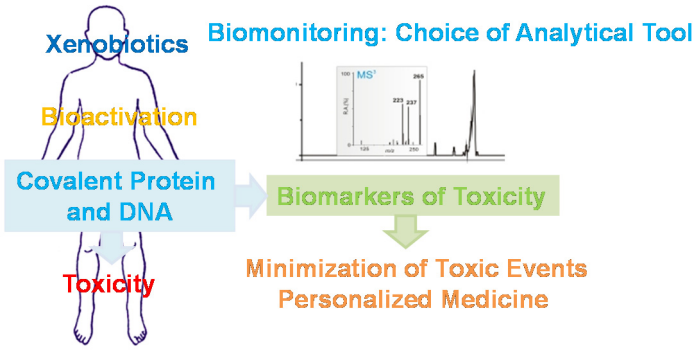
As a chemical toxicologist, my work is aimed at understanding the molecular basis of the toxic events induced by chemical agents from endogenous, environmental, drug, diet and occupational exposure sources. A multidisciplinary approach, founded mainly on solid synthetic and analytical skills, is used within our research group to address the role of bioactivation at the onset of toxic events induced by xenobiotics. Focus have been placed on the establishment of covalent adducts formed with proteins (adductomics), directed towards risk assessment of drugs used in chronic therapies and the development of new biomarkers of exposure to chemical carcinogens. [1-5]



The distinct issues ruling the choice of the most suitable analytical methodologies to monitor these biomarkers of toxicity *in vitro* and/or *in vivo* will be discussed.

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IC-5

ANALYTICAL CHEMISTRY SUPPORTING PHARMA INDUSTRY

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During the past three decades Analytical Chemistry has become an unconditional support in the pharmaceutical and biomedical industry from the drug discovery, during the development process up to the manufacturing.
Classical wet chemistry analysis, chromatography and most recently physical characterization analytical methods give a critical support from the early stages of the productive process, starting on the evaluation of the quality of the raw materials used on the production of the molecule of interest, continuing with the support to the production 1) to determine the period of time required for a reaction step to be completed in order to obtain the highest product yields, 2) to control the formation of undesired impurities with the purpose of obtaining products with the highest purity as well as 3) to monitor and establish the period of time required to remove the solvents used in the production process, solvents that could promote secondary reactions, and that according to industry guidelines should be present below established limits for safety reasons.
After the production of a pharmaceutical form it is essential to demonstrate that the quality of the produced material fulfills the established specification. At this point, the Quality Unit has a key role.
In order to ensure the quality of the produced material till it reaches the final patient, it is necessary to define the shelf life/expiry date under the correct packaging materials and best storage conditions. This is obtained through several years of stability studies.
In the pharmaceutical industry the control of the product's quality must be carried out throughout their complete life-cycle (expiry date/shelf life). This is also assured by continuous stability studies.
Accuracy and reliability of the analytical results are crucial for ensuring quality, safety and efficacy of pharmaceuticals. Incorrect results can lead to release of products that can affect people's health, implying the product's recall from the market with the corresponding financial and reputational implications for the companies.
Quality and reliable analytical data is sustained by three mainstays: instrument qualification, analytical method validation and training. These three points are good manufacturing practices, largely inspected by regulatory entities such as FDA.

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IC-6

ENVIRONMENTAL PROTEOMICS: APPROACHES AND LIMITATIONS

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Nowadays, the changes in the worldwide environment due to environmental stress as pollution or climate change, or even a combination of both, are among the main priority issues for scientific community and predicting outcomes constitute a major challenge for modern society[1, 2].

In this sense, there is an increasing awareness that the analysis of an organism's proteome allows the detection of a total set of proteins' change in response to environmental stress effects. Therefore, changes in the proteome may reflect the levels of contamination or the effects from climate change, especially in the aquatic biota, thus proteome pattern might represent a new diagnostic paradigm in environmental assessment[3].

The most common techniques used in environmental proteomics are 2D electrophoresis and protein identification by mass spectrometry. Typically, the workflow includes several sequential steps: 1) sample preparation, 2) protein denaturation and reduction, 3) protein (peptide) separation, enzymatic digestion and mass spectrometry analysis and (4) bioinformatics and protein identification[4,5]. As such, the "omics", and particularly proteomics, have the potential to provide new insights into the integrative functional responses of organisms to environmental stresses.

Despite the limitations, proteomic studies based on mass spectrometry have been successful in discovering new insights into the complexities of biological systems[6]. The complexity and diversity of marine taxa makes marine proteomics a huge challenge for the decades to come, but it could greatly benefit from advances expected in the near future. Thus, we critically discuss the main aspects related to environmental stress that affect marine organisms, with a special emphasis on the typical proteomic workflow used for marine organisms, the most relevant studies, the main problems and future challenges.

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IC-7

PB ISOTOPES A TOOL IN BIOGEOCHEMICAL STUDIES

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Intertidal sediments are highly structured chemical environments that in the hypothetic absence of physical disturbance consist in layers parallel to the sediment-water interface. Benthic organism and rooted plants disrupt this simple structure by burrowing into the sediment or by growing roots. Their activity affects the rates of transport of gases, solutes and particulate matter within the sediment and between the sediment and the overlying water. This processes as been widely studied using several tools by quantifying interpretative parameters in the water, solids or pore-water. Benthic flux chambers have also been used to quantify the bioturbation rates of some environments. Trace elements have been used with some success but reaction kinetics of early diagenetic processes is always a major critic to their use. On the other hand, stable Pb isotopes are little affected by kinetic processes occurring between the source and sink. Lead has four stable isotopes: 204Pb, 206Pb, 207Pb and 208Pb. The last three are final end-members of the 238U, 235U and 232Th decay chains. The Pb isotope ratios vary with the anthropogenic emissions due to the variety of lead ores used to produce lead additives in gasoline, coal burning and industrial activities. The way that benthic organisms and salt marsh plants influence the Pb signature in sediments will be presented and compared with obtained data of metal concentrations.

IC-8

NEW NANOSTRUCTURED ELECTROCHEMICAL BIOSENSORS FOR ANALYSIS OF BEVERAGES

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The use of redox/conducting polymers together with carbon nanotubes (CNT) is an active area of research, their combination leading to hybrid nanocomposites with superior electrical conductivity and mechanical strength [1-3]. Moreover, biosensors based on such nanocomposites have shown excellent analytical properties [1, 3]. New electrode architectures based on three redox/conducting polymers, i.e. poly(brilliant green) (PBG), poly(methylene blue) (PMB) and poly(3,4-ethylenedioxythiophene) (PEDOT), co-immobilized together with multiwalled carbon nanotubes (MWCNT), were developed in different configurations, to be used as substrates for enzyme immobilization. The electrochemical characterization of the modified electrodes is useful to establish the contribution of each component and for choosing the best configurations for biosensor fabrication.

Enzymatic biosensors with the enzymes glucose oxidase (GOx), alcohol oxidase (AlcOx), alcohol dehydrogenase (AlcDh) and superoxide dismutase (SOD) based on such nanocomposites were developed. The role of dissolved oxygen on biosensor performances will be discussed and the analytical properties of the biosensors will be presented and compared with biosensors based on other nanostructured platforms. Interferences were assessed and stability studies were performed using the biosensors with best analytical performance. The newly developed AlcOx and AlcDh biosensors were used for the determination of alcohol content in different beverages with success, the values being in excellent agreement with those of the producers. The SOD based biosensors were successfully used for the determination of the antioxidant capacity of beverages such as red and white wine, Port wine and berry juice.

Perspectives for the further, future use of these nanomaterial modified enzyme biosensors in the electroanalysis of foods and beverages will be indicated.

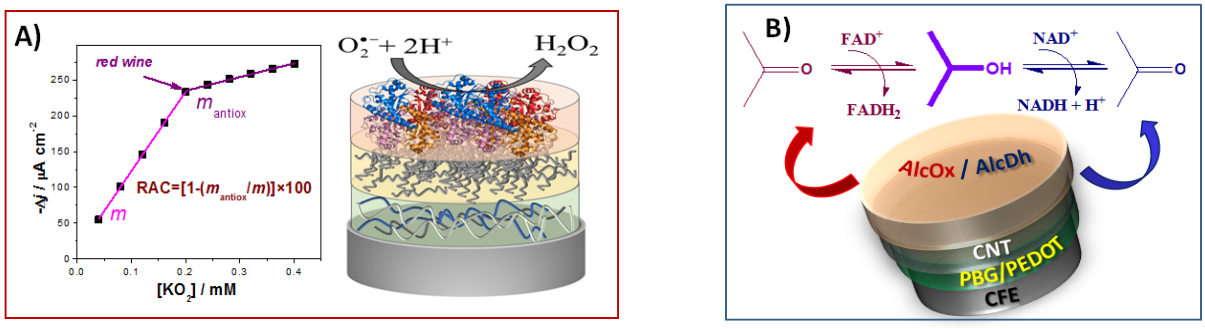

Figure 1: Schematic representation of A) SOD and B) AlcOx and AlcDh biosensors / mechanism.

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IC-9

NEW WET AND DRY ANALYTICAL METHODOLOGIES FOR COFFEE ANALYSIS

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Some recently developed wet and dry analytical methodologies will be presented, covering raw coffee characterization, roasted coffee blends assessment and the quantification of coffee compounds with direct/indirect organoleptic impact during both the roasting process and in the final product.

The developed wet analytical methodologies were based on low pressure flow systems featuring on-line sample preparation strategies. In this context, the exploitation of low pressure chromatographic flow systems [1,2] and the development of a novel automatic solid liquid extraction chamber [3] will be illustrated. The analytical strategies developed for the coffee compounds selected as case studies, besides being good alternatives to the existing methodologies, present as main advantage their general purpose potentiality to be used for other matrices.

The developed dry methodologies were based on near-infrared spectroscopy and chemometrics. The exploitation of these techniques is particularly attractive for coffee companies, due to their main virtues namely no sample preparation, low cost analysis, easy set-up and, easy handling. The case studies selected in this context were the quantification of green coffee beans defects within a coffee batch [4]; the in-line monitoring during the roasting process, of titratable acidity [5] and sucrose content [6] and; the authenticity evaluation of roasted coffee blends [7].

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IC-10

ASSESSMENT OF HUMAN NEUTROPHILS' VIABILITY BY MULTIPLE ANALYTICAL APPROACHES

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Neutrophils, also known as polymorphonuclear leukocytes (PMN), are the most common type of leukocytes, comprising about 70% of all white blood cells. In the event of inflammatory processes, neutrophils display increased mobility, tissue influx ability, prolonged life span, and an increased phagocytic capacity, constituting the initial participants in the cellular defense of the organism.

Due to these important roles, neutrophils are commonly used as an *in vitro* cellular model to evaluate anti-inflammatory and/or pro-inflammatory effects of different compounds, as nanoparticles, metals, plants extracts, and bioactive compounds. Cellular viability assays are mandatory in these studies. There are several methods routinely used in the laboratory to assess neutrophils' viability. Nevertheless, information in literature comparing the different methods in what concerns the viability markers evaluated, time-consumption, the ease of handling and possible interferences, is very scarce.

In this work were tested different viability methods to evaluate the toxic effect of silver nanoparticles on human neutrophils, namely trypan blue, propidium iodide and the neutral red.

The results obtained showed a high variability, depending on the viability test used. Among the studied methods, propidium staining method was the most accurate, rapid and reproducible test.

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ORAL COMMUNICATIONS

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OC-1

MERCURY AND METHYLMERCURY DYNAMICS IN SEDIMENTS ON A PROTECTED AREA OF TAGUS ESTUARY

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Sedimentary dynamics and bioavailability of mercury (Hg) have been actively studied in estuarine ecosystems. Nonetheless, few studies have investigated the processes, fate and bioavailability of Hg in low Hg contaminated areas. With this respect, we have investigated Hg dynamics in two areas inside Tagus estuary natural reserve (RNET, a RAMSAR site), namely Alcochete (ALC) and Vale Frades (VF). Sediment cores were collected during summer and winter periods and pore waters were extracted from solids by centrifugation. Both fractions were analysed for Hg, methylmercury (MeHg) and other interpretative parameters to establish site-specific relationships between them and Hg and MeHg.

Results showed that particulate Hg and MeHg concentrations in both sites were below 1 μg g^{-1} and 4.4 ng g^{-1}, respectively. In both solids and pore waters higher proportion of MeHg was observed in summer months, when the percentage of dissolved MeHg ($MeHg_D$) reached 95% of total dissolved Hg (THg_D), particularly in ALC. In summer, Hg methylation was mainly influenced by sulphate (SO_4^{2-}) and organic matter (OM) presence in ALC, whereas in VF only SO_4^{2-} seems to control the MeHg production. Though some influence of pH and redox conditions was also observed. On the other hand, in the cold season the reduction of Mn(IV) to Mn(II) seems to account to MeHg production in the upper layers, increased the Hg available for methylation in both sites. However, the coupling between reactive dissolved Hg (RHg_D), THg_D and dissolved methylmercury ($MeHg_D$) in VF winter cores indicated that Hg availability is the main driver for Hg methylation.

Thus, linking all these factors, Hg and MeHg partition in these low Hg-contaminated sediments results in a complex combination of chemical and biological mechanisms. Based on diffusive fluxes estimations for Hg and MeHg at the sediment/water interface, indicated that ALC behaved as a sink of both Hg and MeHg, while VF behaved as a sink of MeHg but a source of Hg. In spite of ALC and VF are both located in a RAMSAR site of Tagus estuary, they present different mechanisms of methylation due to their morphology and hydrodynamics that were subjected.

OC-2

A DECADE OF BIOMONITORING OF HEAVY METALS AT LISBON

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In 1999 "leaded petrol" was abolished in Portugal and a significant decrease of environmental lead contamination was expected in the following years.
Poplar tree (*Populus*) is known as a good bio-indicator species and its leaves were chosen to track the level of contamination by lead in the air of Lisbon.
As heavy metals in general may cause several diseases in the nervous system, weak the immune system as well as cause kidney dysfunction or lung cancer, it is essential to monitor these metals in the environment, specially in the big cities where car traffic is a very important anthropogenic source.
Started in 1998, the analysis of lead content in the poplar tree presented a significant decrease in the five years that followed the abolition of "leaded petrol", staying almost unchanged thereafter [1].
Figure 1 shows the results of lead content in Poplar leaves collected in Marquês de Pombal between 1998 and 2011.

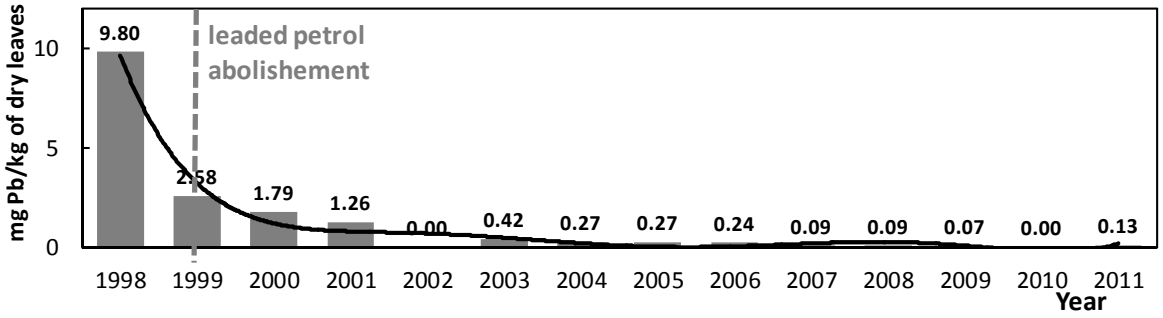

Figure 1 – Lead levels in Lisbon city center (Marquês de Pombal) for 14 years.

In this work, poplar's leaves were collected in six locations of Lisbon, with different car traffic characteristics: city entrances (Calçada de Carriche and 2ª Circular), residential areas (Belém and Cabo Ruivo), city center (Marquês de Pombal) and the green Park of Monsanto, obeying to a well-defined sampling program.
Lead (Pb), cadmium (Cd), nickel (Ni) and chromium (Cr) levels were determined by graphite furnace atomic absorption spectrometry (GFAAS).
The results show that Pb ranged from (9.80 to 0.067 mg /kg of dry leaves) in Marquês de Pombal and from (0.116 to 0.058 mg/kg) in Cabo Ruivo; Ni present values from 2.088 mg/kg in Marquês de Pombal to 0.354 in 2ª Circular; Cr shows a small decrease until 2007 and then a slight increase in every location while Cd presented a similar average value in all the sampled sites.

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OC-3

EXPLORING NEWLY SYNTHESIZED IRON CHELATORS FOR DEVELOPMENT OF *IN SITU* DEVICES

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The environmental monitoring of water samples requires real-time determinations. In order to prevent and anticipate a pollution problem, as well as achieve an appropriate real-time response, frequent analysis is essential. To cope with this requirement, low cost devices, preferably for *in situ* determination, are extremely useful as effective monitoring tools.

In this context, two analytical approaches were explored for the determination of iron(III) in natural waters: the development of microfluidic paper-based devices and ion selective electrodes. Furthermore, to attain a more sustainable chemistry, low toxicity reagents were targeted as alternative reagents.

The aim of our work follows a recently finished project in which especially designed iron chelators were explored in a wet chemistry solution approach [1-3]. The ligands of the 3-hydroxy-4-pyridinone (3,4-HPO) class are synthetically versatile, bear two oxygen coordinating atoms and consequently show a high capacity to trap iron(III) in the form of FeL_3 complexes and a significantly lower affinity for iron(II), a key feature to attain iron speciation. Rhodamine-based ligands were also used for the spectrophotometric and fluorimetric determination of iron (III). The versatility of the used ligands encouraged its further exploring through other approaches.

Microfluidic paper-based devices (μ-PADs) have been gaining an increasing role in environmental monitoring as they are portable, disposable, easy to use, rapid and low cost.

In this communication, the potential of these approaches for in situ applications in environmental monitoring will be discussed.

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OC-4

EXAMINOLOGICAL SOUND CRITERIA FOR GC-MS AND LC-MS IDENTIFICATIONS

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Conventional low resolution GC-MS and LC-MS are the most frequently used instrumentation for the identification of trace levels of organic compounds in various matrices. The compounds identification is based in the agreement between retention time (or relative retention time) and mass spectrum ion abundances ratios of analyte from calibrators with samples peaks. The identification/examination reliability is based on the adequate definition of criteria for these parameters.
Although retention time has an approximate normal distribution, the relative retention time and ion abundances ratios are not normally distributed since result from the combination of two highly correlated variables (e.g. analyte and internal standard retention times or pair of analyte fragments abundances).
This work presents the definition of ion abundance ratios confidence intervals from the estimated dispersion and correlation of abundance values, and the Monte Carlo simulation of abundance ratios.
A user-friendly MS-Excel spreadsheet was developed for the easy definition of identification criteria from previously collected analyte signals of calibrators. The developed methodology was successfully applied to the identification of trace levels of chlorpyriphos methyl and malathion in foodstuffs of vegetable origin [1].

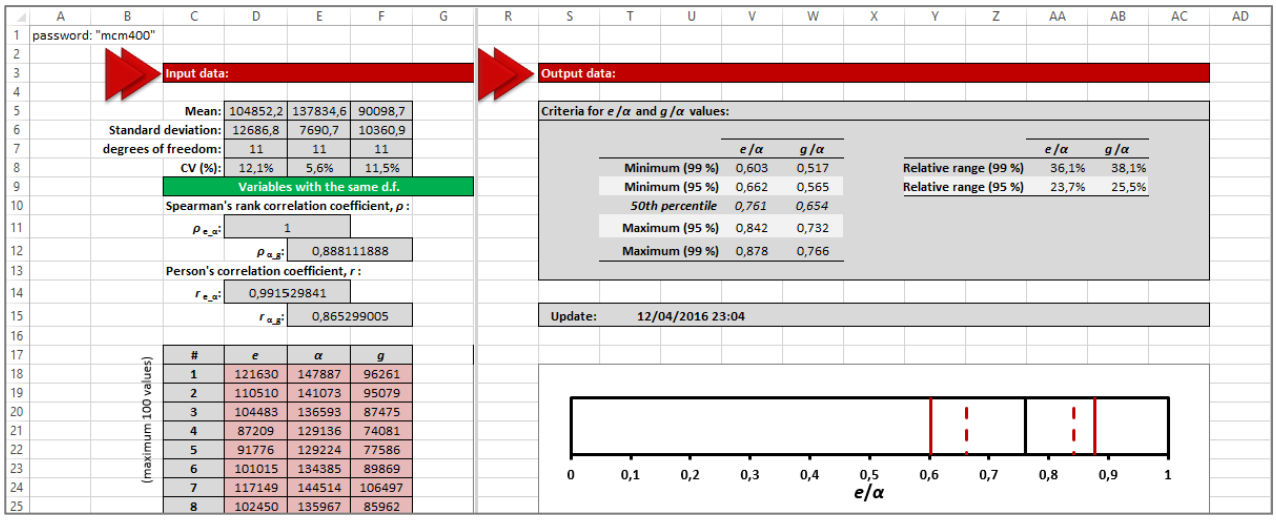

Figure: Pint Screen of developed spreadsheet for the definition of ion abundance ratio criteria.

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OC-5

CONSIDERATIONS ABOUT MEASUREMENT UNCERTAINTY EVALUATION IN ANALYTICAL CHEMISTRY

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The "Guide to the expression of Uncertainty in Measurement" (GUM) [1] establishes general guidance for evaluating and expressing uncertainty in measurement consisting of the main stages: formulation, propagation and summarizing [2]. More precisely, once defined the output quantity, i.e. the measurand, and determined the input quantities, upon which depends the measurand, the formulation develops a model between the former and the latter, assigning probability density functions (PDFs) to the former, on the basis of available knowledge. The propagation of these PDFs through the model enables to obtain the PDF of the measurand leading to an estimate, with associated standard uncertainty and a coverage interval [2].

However, such guidance applies as well as to a physical law or a quantity definition as to a measurement process, e.g. to a calibration model. Consequently expressing the measurement uncertainty as quadratic sum of uncertainty components [3] like:

$u = \sqrt{u_{\text{calib}}^2 + u_{\text{st.dev.}}^2}$, where u_{calib} stands for uncertainty calibration and $u_{\text{st.dev.}}$ bases on the standard deviation of repeated observations, without explaining its origin may lead to erroneous expression of the measurement uncertainty. Indeed, determination and use of straight-line calibration functions are nowadays well documented [4] following the new calibration concept defined in the International Vocabulary of Metrology, consisting on using a mathematical relationship established in the first step of the calibration [5]. A consequence is the possibility to discuss the effects of correlation. The purpose of this communication is to evidence more rigorous practice for measurement uncertainty evaluation in chemistry, taking examples in the fields of solution chemistry such as preparation of a sodium hydroxide calibration standard, titration of a hydrochloric acid solution against the prepared sodium hydroxide solution and calibration of liquid refractometer.

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OC-6

SENSING OF AN ANTIMYCOBACTERIAL AGENT THROUGH AN ELECTROCHEMICAL PORTABLE DEVICE

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The aim of this work is to exploit the versatility of screen-printing technology in the production of disposable screen-printed electrodes (SPEs) intended to the electrochemical sensing of a wide range of substances [1]. The use of SPEs-based portable sensors on the analytical quantification of pharmaceutical compounds in complex matrixes can provide important advantages, such as no pre-treatment steps, great sensibility, simplicity, disposability, cost effectiveness and potential for mass production [2]. Herein, we report an electrochemical sensor based on modified screen-printed carbon electrodes (SPCEs) for the study of the voltammetric behaviour of ethambutol, a first-line antimycobacterial agent used to treat tuberculosis. Cyclic voltammetry and square wave voltammetry were used to investigate the electrochemical behaviour of the drug at the surface of the developed Nafion/multi-walled carbon nanotubes (MWCNT) modified-SPCEs. Moreover, electrochemical impedance spectroscopy and scanning electron microscopy were used to characterize the modified surface of the electrodes. Compared to both unmodified and MWCNTs-modified SPCEs, negatively charged Nafion/MWCNT/SPCEs enhanced the electrochemical sensitivity and selectivity for ethambutol, allowing the detection of the anti-tuberculosis agent in human blood serum and human urine with no pretreatment steps. Furthermore, the produced electrodes provided excellent biocompatibility, good electrical conductivity, low electrochemical interferences and a high signal-to-noise ratio.

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OC-7

ANALYTICAL PERFORMANCE OF *Pseudomonas aeruginosa* BASED BIOSENSORS FOR ACRYLAMIDE DETERMINATION IN WASTEWATER

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Measurement of acrylamide in wastewater discharged by the WWTP (Wastewater Treatment Plant) is a major challenge because there is no reliable and quick method for this analytical measurement [1]. In this study we compare the analytical performance of several biosensors using potentiometric transduction (non-disposable) and conductometric transduction (disposable), developed for acrylamide determination in wastewaters [2].
In the potentiometric biosensors, the transduction element consisted of an ammonium ion selective electrode whereas in conductometric devices the analytical signal was measured by the conductance of the device. The biological recognition element consisted of whole cells of *Pseudomonas aeruginosa* with intracellular amidase activity, immobilized directly on the biosensor's sensitive zone using glutaraldehyde. The biosensors' detection mechanism relied on the monitorization of potential differences or conductivity changes due to the formation of ammonium and hydroxide ions, resulting from acrylamide hydrolysis catalysed by amidase [3].
The best potentiometric biosensor exhibited a sensitivity of 56.6 ± 1.7 mV/decade, a detection limit of 3.35×10^{-4} M, a response time of 3.1 ± 0.2 minutes and a half-life time of approximately 100 days. On the other hand, the conductometric device with the best analytical performance was the one assembled with interdigital silver electrodes deposited form metallic vapour and showed a sensitivity of 123.5 mS/M, a detection limit of 1.34×10^{-4} M and a response time of 1.8 ± 0.3 minutes. The developed biosensors were tested in the determination of acrylamide in wastewater samples collected from a wastewater treatment plant located in the center of Portugal, on different stages of the water treatment. The expanded uncertainties affecting the obtained acrylamide concentrations with both biosensors were estimated and the results were validated by comparison with those obtained with HPLC.

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OC-8

COMPARISON BETWEEN PLS AND OPLS TO BATCH PROCESS MONITORING OF COCRYSTALLIZATION PROCESSES

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Batch processes are widely used in a variety of sectors such as pharmaceutical, petrochemical or life sciences industry [1]. In the pharmaceutical industry the majority of the processes are batch processes. One of the key issues of the PAT initiative is process understanding and control. Batch processes are more difficult to monitor due to their dynamic nature, finite duration, non-linear response and batch-to-batch variability [1, 2]. By using latent variables methods such as principal component analysis (PCA) and partial least squares (PLS) to monitor batch processes, some of this difficulties can be overcome. Multivariate statistical control charts such as Hotelling's T^2 and SPE can then be constructed to monitor the batch process in real time [3]. An alternative to PLS is to apply orthogonal projections to latent structures (OPLS) method. OPLS focusses the analysis on separating the data in two parts: one part correlated with the variable under study and one part with the remaining information (orthogonal). And therefore, interpretation of the model will be simpler [4].

In this work, orthogonal partial least squares (OPLS) is compared with PLS to control a cocrystallization process between hidrochlorathiazide and p-amicobenzoic acid that was monitored on-line using near infrared spectroscopy (NIRS).

Results show that the separation of different types of variations in OPLS lead to different control limits in the scores and Hotellings T^2 control charts. OPLS was able to detect deviations that could not be seen in the PLS-based control charts. Additionally, in several cases, OPLS captured the deviations earlier in the scores and Hotellings T^2 control charts when compared to PLS.

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OC-9

SOILS AND GRAPEVINE LEAVES ANALYSIS BY VISIBLE/NEAR INFRARED SPECTROSCOPY FOR VINEYARD´S SOIL CHARACTERISATION

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The conventional soil survey is often accomplished by means of extensive field observations followed by laboratory analyses, which are extremely time consuming and can be prohibitively expensive. Within the wine community, there has always been particular interest on the influence of the *terroir* characteristics on the features of a wine, but over the past few years a growing interest has spurred on the mechanisms by which a particular soil influences: growth of the vineyard, grape variety characteristics and ultimately wine quality [1]. The need for an efficient, high-throughput analytical method for estimating the impact of soil quality, tillage and thinning on the grapes quality is therefore paramount for the wine industry. Near infrared spectroscopy (NIRS) is a rapid, non-destructive, cost-effective and reliable technique. Its use as a method for discriminating soil types and also for determining different soil constituents is rapidly increasing. It is now considered to be among the most efficient tools for direct *in-situ* analysis of soils, leaves and grapes [2,3]. Two Portuguese vineyards from different wine regions (“Dão” and “Vinhos Verdes”) were monitored. These vineyards had previously been “soil characterized” by pedological methods and were monitored according to designed sampling grids accounting for the specificities of soils and varieties. Soil samples and *Vitis vinifera* leaves were scan *in-situ* using two portable Vis/NIR spectrometers. Spectra were processed through chemometric tools, namely Principal Component Analysis (PCA) and Partial Least Squares Discriminant Analysis (PLS-DA). Preliminary results indicate that NIRS was able to accurately differentiate vineyard soil types. A very high rate of correct predictions was obtained for soil discriminant models based on leaves and soil’s Vis/NIR spectra. Further studies, such as analysis of different maturation stages (regarding leaf analysis) and other geographical regions, are still needed to validate the reliability of this technique. Nevertheless, results indicate that NIRS may prove itself to be a valuable tool for obtaining soil maps and assist in the vineyard’s micro-zoning process. The main advantages over pedological procedures are speed and cost efficiency. It is also an excellent methodology to quickly calibrate hyperspectral satellite images.

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OC-10

MONILITHIC COLUMN APPLICATION FOR REAL TIME MONITORING OF NANOPARTICLES TRANSDERMAL PERMEATION

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Transdermal permeation of active compounds loaded into nanocarriers is currently evaluated in Franz diffusion cells, using pig skin to mimic human skin. Data validity is often affected by the presence of matrix components. Monolithic columns are particularly useful in the determination of analytes embedded in such complex matrices since they present low susceptibility to blockage or back-pressure phenomena, reducing the need for sample clean-up. For this reason, these columns are suitable to be coupled to low-pressure flow systems, affording high throughput, manifold versatility and the use of simple equipment.

In this context, we propose a low-pressure chromatographic system with computer-controlled sampling to quantify nanoencapsulated analytes. The separation of caffeine (model analyte) from matrix compounds was performed using a short monolith along with the spectrophotometric quantification (273 nm). Acceptor liquid collection is simultaneously balanced with the same amount of buffer keeping the pig skin membrane always in contact with this liquid and maintaining the sink conditions.

Several parameters regarding chromatographic analysis were studied along with the establishment of the sampling procedure. The best balance between caffeine separation from matrix components and time of analysis was achieved by using a 15 mm reverse phase monolithic column and a mixture of acetonitrile: water (10:90, v/v) as mobile phase at 0.45 ml min^{-1}. Detection limit was 4 μM and RSD values for caffeine concentration < 2% were achieved. High recovery values were obtained when Hepes buffer incubated as acceptor solution in presence of pig skin for 8 h was spiked with caffeine (103 ± 5 %). The developed system also accounts for low operating costs, low generation of waste and high sample frequencies providing about 48 determinations during two-hour experiments. Sampling can be immediately adapted based on the measured caffeine concentration. Real time sampling and high throughput are compatible with caffeine permeation analysis within the first minutes of contact with pig skin.

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OC-11

PELIMINARY PHENOLIC SCREENING OF *CHAMAEROPS HUMILIS* L. EXTRACTS AND THEIR ANTIOXIDANT CAPACITY ACTIVITY

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Chamaerops humilis L., a medicinal plant which belong to the Arecaceae family, is a shrub-like clumping palm, with several stems growing from a single base [1]. Samples of *chamaerops humilis L* were collected during the year 2015 in Portugal. The leaves were air-dried, powdered and stored for chemical and biological studies.

Methanolic extracts of the dried leaves were examined as potential sources of phenolic compounds Three different methods were used to test the antioxidant activity of the extracts, including colored 2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid radical cation (ABTS*+), DPPH radical scavenging assay (1,1-diphenyl-2-picryl hydrazyl radical reducing power methods). Total phenolic content, in the extracts, was determined according to the Folin-Ciocalteu assay.

The phenolic compositions of the methanolic extracts were elucidated by high performance liquid chromatography coupled on line with a tandem mass spectrometry (HPLC-MS/MS). The extract was mainly composed of *C*- and *O*- flavones and its *O*-methylated derivatives (Figure 1).

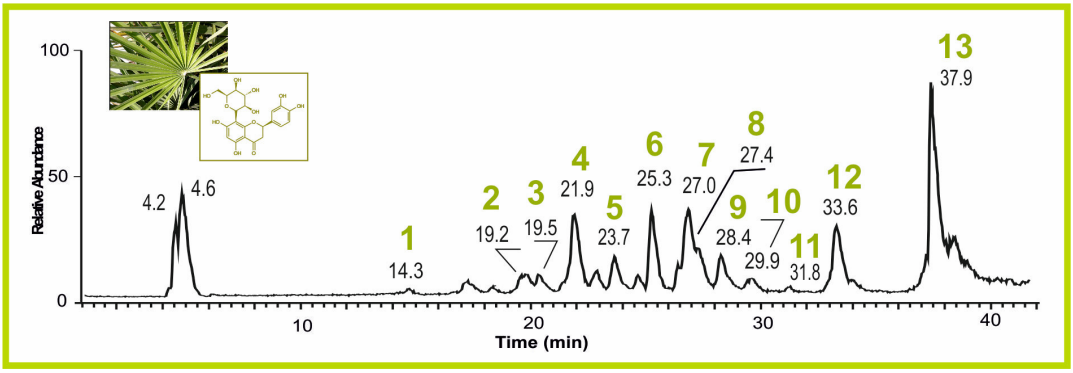

Figure 1: Total ion chromatogram obtained in the ESI negative mode of a methanolic extract of the dried leaves of *chamaerops humilis* L. (1 and 2) 3- and 5-caffeoylquinic acid isomers (MW 354); (3 and 4) procyanidin isomers (MW 578); (5) luteolin-*O*-*C*-glycoside (MW 580); (6 and 7) luteolin 6,8-di-*C*-glycoside isomers (MW 610); (8 and 9) isoorientin and orientin (MW 448); (10) isovitexin-3´´-O-glucopyranoside (MW 594);(11) rutin (MW 610); (12) luteolin-*O*-rhramnosyl-hexoside (MW 594) and (13) tricin-7-*O*-neohesperidoside (MW 638).

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OC-12

ARSENIC, CADMIUM AND LEAD TRANSFER FROM TOBACCO TO CIGARETTE SMOKE: EVIDENCES IN SMOKERS' LUNGS

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Nowadays, about 12% of all deaths among adults aged 30 years and over are attributed to tobacco. It is estimated that globally tobacco kills around 6 million people each year; five million from direct tobacco smoking while 600,000 deaths are attributable to the effects of second-hand smoke [1].

Tobacco smoke is a complex and dynamic chemical mixture. Researchers have estimated that tobacco smoke has more than 7000 chemical compounds from many different classes. Thus, tobacco smoke can be an important source of known toxic compounds such as nitrosamines, polycyclic aromatic hydrocarbons (PAHs), pesticides and metals [2]. Among metal(loid)s, the most commonly associated with health effects are arsenic (As), cadmium (Cd) and lead (Pb). As and Cd are classified as Group 1 carcinogens and inorganic Pb as Group 2A by the International Agency for Research on Cancer [3]. All metal(loid)s in tobacco transfer at some extent into tobacco smoke. However, this transfer rate varies greatly depending on several factors such metal(loid) properties, their concentration in the tobacco, filter type, cigarette design, ventilation and others [2].

In this study, we determine the current content of As, Cd and Pb in cigarette and cigarette ash, in order to calculate the percentage transfer of As, Cd and Pb from tobacco to cigarette smoke and establish a link between smoking and the content of As, Cd and Pb in lung tissue.

The twenty best-selling cigarette brands in Portugal were analyzed for their content in As, Cd and Pb. The decreasing order of metals content was: Cd (0.82±0.19 μg/g) > Pb (0.55±0.07 μg/g) > As (0.14±0.03 μg/g). These values are in good agreement with published data [2, 3]. The highest metal(loid) transference from tobacco to cigarette smoke was observed for Cd (90±2%) and the lowest for As (38±3%). Pb transference was around 53%. Cd and Pb content in smokers' lung tissue was significantly higher than in non-smokers but no significant differences were observed for As.

Cigarettes contains important amounts of As, Cd and Pb and they can pass into cigarette smoke. Lungs show evident signs of smoking habits for Cd and Pb. The significantly higher content of Cd and Pb in smokers' lungs can be attributed to their high transfer from tobacco to cigarette smoke. The presence of these metal(loid)s in cigarette smoke may significantly contribute to its toxic potential, playing a key role in several pathologies.

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OC-13

TRACEABILITY OF THE pH OF SEAWATER - STEPS FORWARD

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pH measurements require prior calibration of the measuring set-up with appropriate pH reference buffer standard solutions. These have pH values assigned by a primary method based on measurements of potential of Harned cells related, through the Nernst equation, with the ionic activities $a_i = \gamma_i . m_i$, (i= Cl^-, H^+). Measured pH values are thus linked to the pH of the reference and to the conceptually defined pH=-lg a_{H+}.

For infinite diluted solutions, approaching ideal behavior, activity equals concentration, with the activity coefficient, $\gamma = 1$. For increasingly concentrated solutions there are significant interactions and activity, *a*, and concentration, *m*, may no longer be assumed to be the same, $\gamma \neq 1$. The Debye-Hückel model of electrolyte solutions with the Bates-Guggenhein convention for Cl^-, valid for ionic strengths below 0.1 mol kg^{-1}, has been used satisfactorily, with an estimated uncertainty of 0.01 (95% confidence). A set of such standard pH buffers is usually supplied with commercial pH meters for calibration of pH measurements [1].

Since seawater, of increasing scientific interest, is a complex electrolytic matrix with ionic strength of approximately 0.7 mol kg^{-1}, recommendations on reference pH buffers have been revised [2]. The validity of the Pitzer model for electrolyte solutions of high ionic strength to the calculation of the mean activity coefficient, $\gamma_{\pm}^{\mathrm{Ptz}}$, has been critically evaluated. An experimental methodology has also been adopted for the assignment of mean activity coefficients , $\gamma_{\pm}^{\mathrm{Exp}}$. Compatibility of $\gamma_{\pm}^{\mathrm{Exp}}$ and $\gamma_{\pm}^{\mathrm{Ptz}}$ values has been demonstrated [3]. Therefore, the Pitzer model can be successfully used to estimate γ_{Cl^-} required in defining the pH reference value of standard buffer solutions with high ionic strength. Tris buffers in synthetic seawater with pH assigned by the primary method have been developed and used in calibration of electrometric measurements. Comparison with pH values obtained by alternative methods, e.g. UV-Vis Spectrophotometry, requires traceability to common references [4]. Measurements in solutions of known chloride ion concentration enable the calculation of the activity coefficients of Cl^- and H^+ and the concentration of H^+, m_{H+}, of the utmost importance namely in speciation studies in environmental complex matrices [5].

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TrainMiC
INTRODUÇÃO À METROLOGIA QUÍMICA

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O programa TrainMiC® de aprendizagem ao longo da vida, http://www.trainmic.org, desenvolvido pelo *European Commission's Joint Research Centre* (JRC), sobre como interpretar os requisitos metrológicos de acordo com a ISO / IEC-17025 para medições químicas e bio-analíticas em diversos sectores (ambiente, alimentar, protecção dos consumidores, etc.), está implementado em mais de 28 países através de equipas nacionais, com mais de 100 formadores reconhecidos pela Comissão Europeia - cada um dos quais assinou um acordo, JRC *User License Agreement,* de utilização do conteúdo do curso TrainMiC®. Estas equipas utilizam ferramentas pedagógicas harmonizadas a nível europeu pelo esforço conjunto de muitos especialistas de toda a Europa através de um conselho editorial. O material encontra-se traduzido em dezasseis idiomas diferentes.
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A equipa TrainMiC® em Portugal (http://trainmic.fc.ul.pt/), composta por Filomena Camões, Cristina Oliveira e Ricardo Silva (CQE-FCUL), Ascensão Trancoso e Paula Teixeira (LNEG), Alice Mosca (A.I.M., Consultadoria, Formação, Auditoria) e Florbela Dias (IPQ) foi constituída em 2006, coordenada, à data, por Filomena Camões, e actualmente por Cristina R. Oliveira. Tem promovido, desde 2007, acções TrainMiC®-PT, em diversas instituições, tanto estatais como privadas. Conta com o apoio da SPQ e realiza anualmente pelo menos uma acção de formação.
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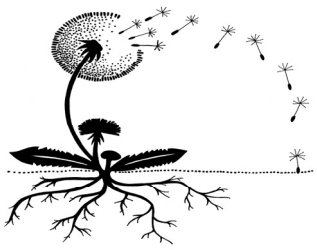

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POSTER COMMUNICATIONS

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P-38	Tutorial and spreadsheet for the evaluation of least-squares calibrations *Cristina Oliveira, Roksana Wójcicka, M. Filomena Camões, Ricardo Bettencourt da Silva*

P-1

Pb^{2+} POTENTIOMETRIC CELL WITH SIMULATENEOUS FLOW OF INNER AND OUTER SOLUTIONS

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Lead is a toxic element exhibiting deleterious effects even at very low concentrations. In this context sensor technologies can provide fast and low-cost determinations for screening or quantification purposes. The unbiased determination of sub-micromolar concentrations of lead by means of potentiometric ion-selective electrodes requires strict control of the analyte concentrations on both sides of the perm-selective membrane phase [1]. The optimization of the membrane composition can also have large effects on the response of the ISE at submicromolar levels and enhance its sensitivity for level trace determinations [2]. In this work, the influence of inner electrolyte solution and composition of ion-selective membrane on the response of Pb^{2+} was investigated. The flow potentiometric cell consisted of two acrylic blocks (5.0 x 3.0 x 2.0 cm - width x height - thickness) with an engraved flow-path. In each block an Ag/AgCl double-junction reference electrode with a 1.0 mol L^{-1} LiCl solution in the outer compartment is positioned in contact with the flow-path and the lead-selective membrane was sandwiched between the blocks. The solutions flow-rate was fixed at 1 mL/min. The ion-selective membrane composition regarding the content in the ionophore (Lead ionophore IV), sodium tetrakis(4-fluorophenyl)borate additive (NaTFPB), dioctyl sebacate plasticizer (DOS) and PVC was optimized to attain nernstian response in the sub-micromolar range. The best results were obtained for membranes formulated with 0.17% (w/w) of the ionophore, 0.01% of NaTFPB, 49.82% of the DOS plasticizer and 50% PVC, which rendered linear calibrations between 10^{-7} and 10^{-4} mol L^{-1} of Pb^{2+} with slope of 30 mV/dec and with a practical detection limit of 10^{-8} mol L^{-1}. In order to overcome irreproducible super-nernstian responses resulting from trans-membrane ion-fluxes a 0.1 mol L^{-1} EDTA in acetate buffer (pH 4.5) solution containing inner flowing electrolyte. A 10^{-7} mol L^{-1} Cu^{2+} flowed between samples to minimize cross-contamination. The sensor performance is now under evaluation considering the determination of lead in biological fluid samples.

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P-2

BAR ADSORPTIVE MICROEXTRACTION (BAμE) - A SIMPLE, ROBUST AND EFFECTIVE SAMPLE PREPARATION TECHNIQUE

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In the last decade, the sample preparation methodologies have been characterized by devices with convenient design and great effectiveness to enhance selectivity and sensitivity, in agreement with the green analytical chemistry principles, for trace and ultra-trace analysis [1].
Recently, a novel microextraction technique was introduced, bar adsorptive microextraciton (BAμE), which represents an effective alternative to monitor trace levels of a wide range of (polar) organic compounds in environmental and biological matrices [2]. BAμE operates under the "floating sampling technology" and, is based on the use of analytical devices, light in weight, simultaneously with a conventional Teflon magnetic stirring bar at the bottom of a sampling flask. When the sample matrix is rapidly spinning around due to centripetal force promoted by the magnetic bar, the analytical device is left under free-floating motion just below the center of the vortex. During a static process, the analytes migrate by diffusion from the bulk sample and become retained in a convenient sorbent phase, where the microextraction takes place. This novel analytical approach has been applied in combination with convenient chromatographic and hyphenated systems mainly for trace and ultra-trace analysis of pesticides, pharmaceutical products, drugs of abuse and food additives in several types of matrices (e.g., water, biological fluids, beverages, etc.) The present contribution is an overview of this novel microextraction technique.

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P-3

FLUORESCENT DETECTION OF NITROANILINES: SUCCESSES AND PITFALLS

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Nitroanilines (NAs) are widely produced for a variety of industrial and commercial purposes, including intermediates in the industrial synthesis of dyes, pesticides, and pharmaceuticals. Their partial release into environment calls for simple, rapid and effective methodologies for their detection and monitoring. Different sensing systems have been developed in the last years [1]. Exploring the inherent capabilities of certain fluorescent calixarene scaffolds for establishing strong host:guest interactions, several sensing materials have been recently developed by us [2].

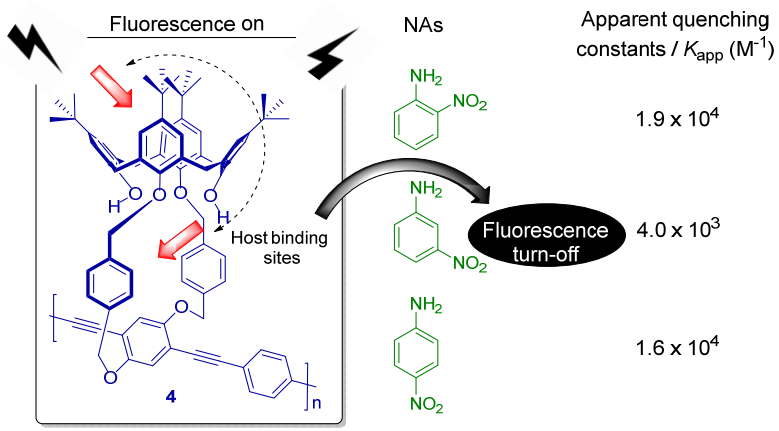

Figure 1: Polymer fluorophore (Calix-OCP-PPE) showing possible sites for guest interactions and quenching efficiencies of nitroanilines in after titration in $CHCl_3$ (λ_{exc}=380 nm).

Herein the sensing abilities of a fluorescent calix[4]arene-based conjugated polymer (Calix-OCP-PPE) toward isomeric NAs are described (Fig. 1). The huge sensory efficiencies shown toward NAs are likely a result of three conjugated quenching mechanisms: i) inner-filter effects; ii) Förster resonance energy transfer and iii) photoinduced electron transfer. Discussion along these topics will be presented.

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P-4

CHARACTERIZATION OF A BIOCATHODE CONSISTING OF LACCASE FROM *RHUS VERNICIFERA* IN MODIFIED GRAPHITE SUPPORT

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Lately, biosensing research is been focusing in miniaturization, portability and energetic autonomy. One of the problems associated with portability is the power supply. Katz and his team (2001)[1] were the first to overcome this drawback by creating a self-powered biofuel cell able to generate 1uW of power with signal intensity being nernstian related with analyte/fuel concentration. The biofuel cell for glucose sensing purposes comprised a bioanode immobilized with glucose oxidase and a biocathode immobilized with cytochrome c oxidase, responsible for dioxygen reduction. One way to achieve further miniaturization with reasonable power output is the use of laccase enzyme for air-breathing cathodes. Enzymes with copper-redox catalytic centres, such as laccases, present low selectivity to oxidize phenolic compounds thus evidencing shallow active centres located near the protein surface. This feature indicates direct electron transfer ability for implementation of efficient third generation bioelectrodes. Also, to improve electron transfer efficiency, nanostructured carbon materials are frequently used as wiring between the active site of the biological element and the electrode for electron transfer improvement.

The main objective of the present work is the study of dioxygen electroreduction as well as determination of power density and polarization curves through amperometric methods of a biocathode consisting in a modified graphite electrode with immobilized *Rhus vernicifera* laccase.

The electrodes were implemented with a 2 mm commercial pencil mines over which 10 µL of graphene oxide (1mg/ml) were dropped and further electro-reduced by cyclic voltammetry at 50mV/s in the interval -1.5 to 0.6 V vs. AgCl/Ag, for 33 hours. The graphene surface was then casted with 10µL of a mixture consisting of laccase and carbon nanotubes in silicon titanium tetraglycerolate and left to dry at ambient temperature for about 5 hours. In the presence of oxygen saturated solution of electrolyte a catalytic cathodic current was observed corresponding to dioxygen reduction at the biocathode surface. The response shows good linearity to a maximum concentration of around 0.2 mM with a slope of 4 uA/(mM.cm^2). Amperometric experiments were conducted applying a potential of -0.1V in stationary conditions.

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P-5

RAPID DETERMINATION OF HALOACETIC ACIDS IN DRINKING WATER BY ULTRA-PERFORMANCE LIQUID CHROMATOGRAPHY--TANDEM MASS SPECTROMETRY (UPLC-MS/MS)

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Haloacetic acids (HAAs) are a class of disinfection by-products in chlorinated waters, formed by the interactions between natural organic matter and the disinfectants and are suspected of being carcinogenic and mutagenic. Therefore most attention had been focused on the occurrence and quantification of HAAs in drinking water.

There are a total of nine HAAs, including monochloroacetic acid (MCAA), monobromoacetic acid (MBAA), dichloroacetic acid (DCAA), bromochloroacetic acid (BCAA), dibromoacetic acid (DBAA), trichloroacetic acid (TCAA), bromodichloroacetic acid (BDCAA), chlorodibromoacetic acid (CDBAA) and tribromoacetic acid (TBAA). In terms of current legislation, the USEPA (United States Environmental Protection Agency) has as reference value a limit of 60 µgL-1 for the sum of five HAAs (MCAA, DCAA, TCAA, MBAA and DBAA) [1]. In Portugal, the ERSAR (Entidade Reguladora dos Serviços de Águas e Resíduos) has as reference value the sum of the concentrations of MCAA, DCAA and TCAA that should not exceed 100 µL-1[2].

The most commonly used methods for the determination of HAAs are Gas Chromatography (GC) with electron capture detector (ECD) and GC equipped with mass spectrometry (MS). Both methods require previous extraction and derivatization of the HAAs prior to their determination, being not environmentally friendly, due to the large consumption of organic solvents, and quite time consuming.

In this work a method that combines the efficiency of ultra-performance liquid chromatography with MS (UPLC-MS/MS) was implemented and validated for the direct analysis of the five HAAs stated by USEPA. Limits of quantification for the HAAs study were below the reference values imposed by USEPA and ERSAR. Different drinking water samples were then analyzed and in none of the samples were exceeded the reference values.

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P-6

FRUIT EXTRACT FLAVONOIDS ELECTROCHEMICAL DETECTION AND ANTIOXIDANT CAPACITY EVALUATION

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Natural phenolic derivatives are compounds that, in low concentration, can provide health benefits by preventing biomolecules (proteins, nucleic acids, lipids, sugars) from undergoing oxidative damage through free radical mediated reactions.

Flavonoids in different fruits: nectarine, prickly pear cactus, apricot, sour cherry, European and Japanese plum, pomegranate, and date, using an ultrasound assisted extraction method, were extracted.

The detection of flavonoids in the fruit extracts, by RP-HPLC coupled with electrochemical (EC) and photodiode array (PDA), following programmed sequences of isocratic or gradient elution, was carried out. Flavonoid standards were used to identify the flavonoid contend in the fruit extracts.

Scheme 1: Antioxidant activity evaluation and characterization by DPV and DPPH● assay.

The fruit extracts total antioxidant activity, *i.e.* total phenolic content, by differential pulse voltammetry (DPV), at a glassy carbon electrode, considering the oxidation current and potential (electrochemical index-EI), and by chronoamperometry at fix potential, was evaluated.

The fruit extracts free radical scavenger efficient concentration (EC_{50}), using the DPPH● assay, was determined and compared with the catechin standard.

The results showed the excellent sensitivity of the electrochemical detection, its suitability for the detection of low levels of electroactive phenolic compounds in fruit extracts, and allowed the determination of much lower concentrations of the analyte, without interferences, comparing with the spectrophotometric detection.

P-7

CONSTRUCTION OF AN IRON(III) SELECTIVE ELECTRODE WITH A RHODAMINE-BASED NEWLY SYNTHESIZED CHELATOR

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The concentration of iron in natural waters is an important indicator of the water quality. As a micronutrient, iron(III) has a particularly important role in the environment as shown by the cycle of iron. The variation of physical-chemical parameters of the water body can shift the oxidation state of iron. The impact of this process may cause irreversible environmental damage.

A rhodamine-based turn-on fluorescent ligand has been especially designed to complex iron(III) in physiological applications [1]. In addition to their high iron (III) affinity, its low solubility in water potentially makes it potentially suitable for the preparation of ion-selective membranes. In this scenario, different cocktail compositions were used for the preparation of the electrode plastic membrane. The resulting electrodes performance was evaluated in terms of sensitivity, response time and potential interferences; these results are presented and discussed in this presentation.

The combination of these ligands with potentiometric detection intended to add the advantage of portability, possible with ion selective electrodes, with the ligands selectivity. In this context, we aim to apply the iron(III) electrodes to the in situ determination of iron in natural waters.

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P-8

OCEAN ACIDIFICATION – pH AND ALCALINITY OF SEAWATER

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The continuous exchange of CO_2 between the atmosphere and the seawater and hence their capacity to absorb atmospheric CO_2 make the oceans to be recognized as a carbon sink. Due to its solubility in water, CO_2 rapidly forms carbonic acid, H_2CO_3, starting a series of proton transfer reactions to form bicarbonate, HCO_3^- and carbonate, CO_3^{2-}. Under seawater pH conditions (≈ 8) excess of CO_2 added to the ocean reacts with carbonate ion to form bicarbonate ion without utilizing the hydrogen ion:

$$CO_2(g) + CO_3^{2-} + H_2O \rightleftarrows 2\ HCO_3$$

Changes in acidity affect the various simultaneous homogeneous and heterogeneous equilibria of the carbonate system and of metals' speciation, hence the bioavailability of the respective chemical elements.

Seawater carbonate system is described by four parameters linked through thermodynamic constants: Total alkalinity which refers to the amount of acid needed to neutralize bicarbonate and carbonate ions (TA), pH, Dissolved Inorganic Carbon (DIC), CO_2 fugacity (or CO_2 partial pressure, *p*CO2). By measuring two or more parameters, e.g. pH and TA, it is possible to calculate those not measured [1]. However, serious problems of lack of metrological traceability, hence comparability, of measurement results are highlighted by the oceanographic community. Metrological tools are requested in order to be able to measure large composition changes as well as to detect subtle changes and discriminate between the effects of anthropogenic CO_2 due to human activities and the processes that are internal to the ocean. Therefore, the entire measurement chain needs to be explored.

This work aims at providing a traceability framework for the measurement of different oceanographic observables related to the carbon cycle. Due to the high complexity of the saline matrix (Ionic strength, $I \approx 0.67$ mol kg^{-1}), pH measurements of seawater samples required adequate reference calibration pH buffer solutions [2]. Tris-Tris HCl buffers solutions in artificial seawater background were used as traceable standards for the potentiometric pH measurements [3, 4]. Titrations with 0.1 mol kg^{-1} HCl were conducted in the same samples [5] in order to measure TA.

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P-9

A NOVEL ELECTROCHEMICAL SENSOR FOR BISPHENOL A BASED ON CARBON NANOTUBES/GOLD NANOPARTICLES MODIFIED GLASSY CARBON ELECTRODE

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During the past few years, environmental chemists have been widely concerned about endocrine disrupting chemical substances. Among them, bisphenol A (BPA) is highly suspected to act as an endocrine disruptor and is used mainly in the landfill leachates, milk bottles, paper, packaging and plastic plants. Some studies suggest that BPA also exhibits an adverse effect on the human immune function, causing reproductive disorders including decrease of sperm quality in humans, birth defects due to fetal exposure and various kinds of cancer, for example: prostate, testicular and breast cancer [1]. The determination of BPA has been carried out directly or indirectly by enzyme inhibition using a variety of electrochemical methods: differential pulse voltammetry, cyclic voltammetry, amperometry, etc. Modification of the electrode surface has been demonstrated to be effective for improving the sensing performance of bisphenol A. The modifiers include: graphene nanofibres and gold nanoparticle (AuNP) composite modified glassy carbon electrode (GCE) [2], chitosan–Fe_3O_4 nanocomposite modified GCE [3], porous polymerized ionic liquid film GCE [4], etc.

In the present work, a novel electrochemical sensor based on carbon nanotubes (CNT) and AuNP composite modified glassy carbon electrode has been developed. Experimental parameters, such as the scan rate and the pH value of the buffer solution, were first optimized. Different electrode configurations with CNT and AuNP at various loadings have been investigated for BPA detection. It was observed that the lowest limit of detection was achieved when the CNT loading was the lowest from those tested; however, the sensitivity increased when increasing the amount of AuNP. The reproducibility, repeatability and stability of the sensor have been examined and comparison with literature using similar architectures for BPA made. Finally, the selectivity and practical application of the developed modified electrode for the determination of BPA has been verified.

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P-10

$\delta^{13}C$ RECORDS IN DOG COCKLE (*Glycymeris glycymeris*) SHELLS AS A PROXY OF SEASONAL IBERIAN UPWELLING

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Bivalve shells provide robust high-resolution archives of oceanographic and climatic variability on timescales that can extend to before the period with instrumental records. In particular, the North Atlantic Ocean region has recently seen several noteworthy sclerochronological and geochemical reconstructions based on bivalve shells of high frequency oceanographic and climatic conditions during the last millennium. However, sclerochronological studies of southern European coastal shelf seas are limited by the absence of long-lived bivalve species (i.e. several decades to hundreds of years) The dog cockle *Glycymeris glycymeris* (with longevity > 100 years) may be a novel and suitable archive for past environmental conditions in the Iberian Upwelling System (IUS), the northern section of the Canary Current Eastern Boundary Upwelling Ecosystem.

This project aimed to evaluate the suitability of *G. glycymeris* as an archive of past upwelling conditions in the IUS. Here we assess the potential of carbon stable isotope ($\delta^{13}C$) shell records using two approaches: (1) a sub-monthly sampling, allowing a high resolution record between 2001 and 2007 and (2) annually-resolved samples from 1955 to 2013. Sub-monthly, the variation of shell $\delta^{13}C$ was inversely synchronous to upwelling intensity, likely explained by seasonal changes in upwelling-related ventilation of surface waters with lower $\delta^{13}C_{DIC}$ subsurface waters, i.e. higher upwelling during spring-summer lowering surface water $\delta^{13}C_{DIC}$, and thus lowering shell $\delta^{13}C$.

The annually-resolved records showed a marked and stable ontogenetic decreasing trend in $\delta^{13}C$. However, removal of the ontogenetic trend produced a mean annual $\delta^{13}C$ index which had a similar variation to the inverted amplitude in upwelling intensity (AUI) between the high and low upwelling seasons (JAS-DJF). A potential mechanism is proposed where AUI changes the seasonal growth bias in the seawater $\delta^{13}C_{DIC}$ signal integrated by annual shell $\delta^{13}C$. Consequently, high (low) AUI would bias shell growth towards the high (low) upwelling season, and thus lower (higher) $\delta^{13}C_{DIC}$ and ultimately lower (higher) shell $\delta^{13}C$.

Although replication and the extension of $\delta^{13}C$ records are needed to validate the mechanism linking shell $\delta^{13}C$ to UI and $\delta^{13}C_{DIC}$ seasonality, these results support *G. glycymeris* shell $\delta^{13}C$ as a valid proxy for seasonality in the IUS.

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P-11

MICROWAVE-ASSISTED AQUEOUS SYNTHESIS OF CdTe QUANTUM DOTS AS PHOTOLUMINESCENT PROBES FOR DETERMINATION OF METFORMIN

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The preparation of colloidal semiconductor nanocrystal, or quantum dots (QDs), with improved quality in terms of optical and chemical properties has had a significant progress in the last few years. Recently, the aqueous synthesis of QDs assisted by microwave irradiation have been exploited in order to obtain highly luminescent, monodisperse and crystalline nanoparticles for further use as photoluminescent probes in chemical analyses.
In the present work, different sizes of CdTe QDs capped with 3-mercaptopropionic acid (MPA) and glutathione (GSH) were synthesized by using the microwave-assisted aqueous route, which were used as photoluminescent probes for the indirect fluorometric determination of metformin. The proposed methodology involved, in the first step, the interaction of CdTe QDs with Cu(II) ions at fixed concentration, inducing thus a markedly decrease of the nanoparticles photoluminescence. Then, the photoluminescence decrease of CdTe nanoparticles was restrained when adding increasing concentrations of metformin to the Cu(II) solution which allow the quantification of metformin in pharmaceutical formulations.
In the optimization study, the QDs size, pH of solution, the sequence of reagents addition, Cu(II) concentration and the kinetic of the interaction of QDs-Cu(II)-metformin system were thoroughly evaluated.

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P-12

SURVEY OF ANALYTICAL METHODS FOR QUANTIFICATION OF TRANEXAMIC ACID IN PHARMACEUTICALS AND BIOLOGICAL FLUIDS

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Tranexamic acid (TXA, figure 1) is a synthetic derivative of the amino acid lysine developed in 1960's and introduced into clinical practice more than 40 years ago. The antifibrinolytic effect of TXA results from the reversible blockade of lysine binding sites through formation of a reversible complex of the drug with plasminogen molecules blocking the action of plasmin [1, 2]. The usefulness of TXA has been reported namely for women suffering from menorrhagia, bleeding during pregnancy and for prevention and treatment of postpartum haemorrhage, in upper gastrointestinal bleeding, bleeding after cardiac surgery, among others.

The interest on TXA overwhelmingly grew after withdraw of aprotinin in 2008 [3], and a renewed attention has emerged in the literature since the pharmacokinetic, optimum dose and administrations schedules of this drug are still subject of research. Therefore, the quantification of this molecule in both pharmaceutical formulations and biological fluids has been proposed for evaluation of pharmacokinetics and therapeutical assessment. Methods based on spectroscopic detection and ion-pair HPLC have been applied for quantification in pharmaceutical products, providing detection limits ranging from 0.76 to 13 μM [4]. For quantification in biological fluids, particularly blood plasma, lower values must be attained. Hence, more sensitive techniques, such as GC-MS and HPLC-MS/MS have been applied. Taking these into account, the main goal of this work was to review the most used analytical methods for the detection and quantification of TXA, with special focus in biological fluids, in a systematic way and highlighting innovative features of recent methods.

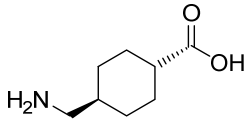

Figure 1: Tranexamic acid.

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P-13

CALCIUM-INDUCED CALMODULIN CONFORMATION CHANGE. ELECTROCHEMICAL EVALUATION

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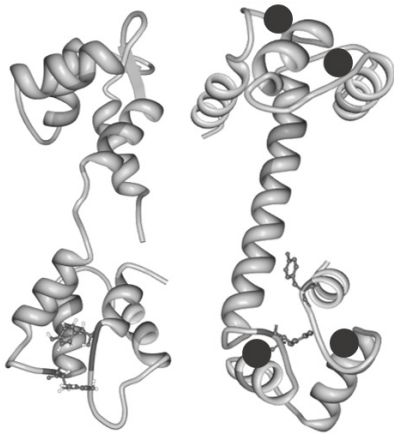
Calmodulin (CaM) is an essential protein present in all eukaryote cells and is one of the most important Ca^{2+} signalling protein, responsible for the regulation of numerous Ca^{2+}-mediated signalling pathways.
Structurally CaM is composed by two domains, N- and C-terminal domains, linked by a flexible central α-helix. CaM can aggregate up to four calcium ions (two Ca^{2+} per domain) and exists in two forms: without calcium (ApoCaM) and in the calcium saturated form (HoloCaM), Scheme 1, changing its conformation and determining how it recognizes and regulates its cellular targets.



Scheme 1: 3D Structure of Apo-CaM (left) and Holo-CaM (right) with calcium atoms (black) [pdb file: 1CFD (Apo-Calmodulin) and 1CLL(Holo-Calmodulin)].

The oxidation mechanism of native and denatured CaM, at a glassy carbon electrode, using differential pulse voltammetry, was investigated. Native and denatured CaM presented only one oxidation peak, related to the oxidation of tyrosine amino acid residues. Ca^{2+}-induced CaM conformational change and the influence of Ca^{2+} concentration in the electrochemical behaviour, was evaluated. Significant differences, in the peak potential and peak current of tyrosine amino acid residues, in the absence or presence of Ca^{2+} ions, were observed. The glassy carbon electrode surface modified with immobilized multilayer CaM film was characterized using electrochemical impedance spectroscopy.
The improvement of effective methods for determination and quantification of CaM is essential for drug development, clinic diagnosis and disease etiology investigation, due to the importance of CaM in the control of numerous physiological processes.
The changes Ca^{2+} induced in CaM conformational structure resulted in an alteration of Tyr amino acid residues electrochemical signals and may constitute the base for the development of a new generation of CaM based Ca^{2+} biosensors.

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DETECTION OF EXPLOSIVES BY CALIX[4]ARENE-CARBAZOLE THIN FILMS: MATRIX AND DEPOSITION METHOD EFFECTS

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Enhancement of homeland security, detection of hidden landmines, environmental detection of explosives and related forensic investigations deeply rely on the development of chemical sensors and devices thereof for trace detection of high explosives [1]. Nitroaromatic compounds (NACs) are found in many explosives' compositions and as thus have been the most targeted group of substances envisaged in research efforts for low-cost, expedite and sensitive portable chemosensor devices. A series of highly sensitive and selective calixarene-based sensing materials have been previously reported by us for the detection of NACs [2] and nitroaliphatic liquid explosives and explosive taggants [3] in vapor-phase. To further enhance the sensing abilities of some of our calixarene-based sensors toward NACs and simultaneously produce low-cost real-operating sensing devices for on-field detection, studies using thin films of calixarene-carbazole conjugates (calix-1) [4], either neat or in several natural and synthetic polymer matrices, were undertaken. Detection of 2,4-dinitrotoluene (DNT; 190 ppb at 25ºC) and 2,4,6-trinitrotoluene (TNT; 10 ppb at 25ºC) at equilibrium concentrations was performed using a static setup in a time-dependent manner. Factors affecting the films' performance, such as the nature of the polymeric matrix and the deposition method (spin-coating *vs* electrospinning) were investigated. Neat thin films of calix-1 obtained from *N*,*N*-dimethylacetamide solutions by spin coating present so far the highest sensitivity to DNT vapors (Fig. 1), with quenching efficiencies (QE) reaching *ca.* 75% within just one minute of exposure (corresponding to a vapor-phase K_{SV} = 4.8 x 10^{-2} s^{-1}). The results obtained heretofore indicate a rather perceptible matrix and/or deposition method dependency.

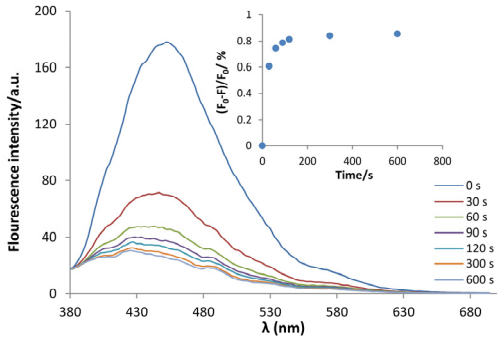

Figure 1: Emission intensities of calix-1 neat film upon exposure to DNT vapors (up to 600 s; top to bottom); inset: fluorescence quenching efficiencies for the neat film of calix-1; λ_{exc}=360 nm.

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P-15

LASER INDUCED FLUORESCENCE (LIF) TECHNIQUE FOR ENVIRONMENTAL APPLICATIONS

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Laser-induced fluorescence (LIF) spectroscopy is widely used for studying properties of molecules able to absorb radiation at one wavelength and re-emitting that radiation at wide range of longer wavelengths. LIF sensors can be developed to detect the spectrum of re-emitted radiation. In contrast with more sophisticated analytical methods, LIF spectroscopy yields nearly instant results and requires neither reagents nor considerable power. These features make LIF techniques extremely useful for environmental applications. The authors have developed a LIF detector on the basis of a commercial palm-size spectrometer (Ocean Optics USB4000) and a reliable and robust solid-state frequency-doubled Nd:YAG laser, and have applied the technique to several environmental monitoring purposes, such as (i) evaluation of the impact of different types of stress, in particular, the drought stress on higher plants - cork oak (*Quercus suber*), maritime pine (*Pinus pinaster*), and genetically modified *Arabidopsis* plants, as well as tracing the impact of trace metal stress on marine microalgae (*Phaeodactylum tricornutum*); (ii) description of the migration of intertidal microphytobenthos; and (iii) assessing and mapping algal communities.

As an indirect method, LIF analysis is complemented by other, more direct or independent measurements, to verify the results obtained. The application of the LIF technique to these environmental studies indicated that, for short detection ranges, the required detection efficiency can be achieved using sufficiently low laser pulse energies, providing reliable and accurate results. The LIF is also extremely time-saving, non-invasive, highly sensitive, and effective alternative tool with potential to reliably assess environmental impacts on different ecosystems. Results obtained from these studies supported the use of LIF-based techniques for environmental sensing and screening, to get a rapid large-scale assessment of an entire region under surveillance and then identify the stressed environments and corresponding zones of high interest, which can be studied in detail afterwards, utilizing more expensive and time-consuming methods.

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DUAL-EMISSION RATIOMETRIC ASSAY BASED ON PHOTOLUMINESCENCE BLUE-EMITTING CARBON DOTS AND RED-EMITTING CdTe QUANTUM DOTS FOR H_2O_2 DETERMINATION

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Hydrogen peroxide is a strong oxidant frequently used in various applications as cleaning agent. Due to its broad antimicrobial activity, H_2O_2 is used for contact lens disinfection. However, H_2O_2 can be toxic to the ocular epithelium and cornea being necessary to perform a carefully monitoring of its concentration in order to guarantee the safety of its utilization.

Photoluminescence (PL) monitoring via a single photoluminescence probe can be affected by several factors, including interfering species, leading to fluctuations on measured signal or to incorrect readouts. In this regard, resorting to a combination of probes, in this case distinct nanomaterials excited at the same wavelength and emitting at different ones, could be used as a ratiometric photoluminescence assay that can markedly reduce the influence of external factors, improving accuracy.

In the present work, two distinct photoluminescent nanoparticles were exploited to implement a ratiometric assay for the detection of H_2O_2 in real samples. This dual-emission nanosystem involved the combination of a blue-emitting carbon dot with a red-emitting CdTe QDs capped with 3-mercaptopropionic acid (MPA). After the mixture of CDs and QDs, the photoluminescence emission of CDs markedly decreases, prevailing the red emitting PL of QDs. The addition of H_2O_2 in the mixture induces the depassivation of the MPA-CdTe QDs, decreasing thus the red-emission PL while the photoluminescence of the blue-emitting CDs increases. This PL changes were used for the determination of H_2O_2 in lens care solutions.

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P-17

PAPER-BASED SENSOR WITH N-DOPED CARBONS DOTS FOR IRON(III) DETECTION

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The development of point-of-care (POC) diagnostic sensors based on nanoparticle is a promising area of research. The hallmarks of POC sensors development relies on simple sensors and cost-effective instrumentation. Photoluminescence nanoparticles provides a simple and selective signal which can be easily measured and quantified by a portable and ubiquitous optical device. In addition, these nanoparticles can be immobilized into solid supports taking the test strips shape [1].

Fluorescent carbon dots (CDs) are a new class of carbon nanomaterials that have harvested increasing interest as alternatives to cadmium-based quantum dots [2]. In addition to their incomparable optical properties, CDs have shown to have low toxicity, biocompatibility and low cost. Moreover, they can be easy engineered either by surface functionalization or by chemical doping with heteroatoms, namely with nitrogen atoms [3].

Herein, we describe a "bottom-up" microwave-assisted synthesis of N-doped CDs using citric acid and ethylenediamine as starting materials. N-doped CDs were obtained with high quantum yield and with short reaction times. Its fluorescence is selectively quenched by the presence of iron(III). As proof of concept, a test strip sensor was built by immobilization of CDs on a paper-based support. Taking advantage of high-resolution camera equipped in smartphone, signal detection and quantification was achieved by recurring to a smartphone.

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P-18

NOVEL EXTRACTION PROCEDURE FOR THE DETERMINATION OF BIOGENIC AMINES IN RUMEN FLUID

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In the ruminant digestive tract, feeds are extensively fermented in the rumen by the microbial population mainly producing volatile fatty acids, methane, carbon dioxide and ammonia. Other volatile compounds such as methyl sulfide, dimethylsulfide, acetaldehyde, amines, alcohols, phenols, aldehydes and ketones, are also produced in smaller amounts. Biogenic amines mostly result from the decarboxylation of amino acids in the rumen [1]. These compounds have been evaluated due to the increasing interest of their putative role in animal health [2].

This work aimed to develop a methodology for the extraction of biogenic amines from rumen fluid, and its subsequent determination by high-performance liquid chromatography (HPLC) with fluorescence and mass spectrometry detection. The extraction of the analytes was performed by volatilization directly from the sample and retained in a solid-phase extraction (SPE) column. The retained analytes were then derivatized by passing an *o*-phthalaldehyde (OPA) solution through the extraction column; the derivatives were eluted with an organic solvent and the eluate was injected in a HPLC system for separation and analysis.

Several parameters of extraction and derivatization of biogenic amines were studied (such as extraction time and temperature, pH and OPA concentration). The methodology was then applied to the identification and quantification of biogenic amines in rumen fluid samples from animals fed different diets.

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P-19

DIESEL ANALYSIS: SETTING UP QUALITY CONTROL FOR DETERMINATION OF FAME CONTENT

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In order to contribute to the decrease of fossil fuel dependency as well as of GHG emissions, the European commission has been promoting the substitution of the traditional fuels used in the transportation sector by alternative fuels. The EU directive 2009/28/CE established a mandatory 10% minimum target of alternative fuels to be achieved by all Member States in transport sector consumption by 2020. Biodiesel (fatty acid methyl esters – FAME), a biofuel derived from vegetable oils and other materials with glyceride content, emerged as a viable option as it can be used as pure or blended with diesel, due to the similarity in their physical and fuel properties. The EU directive 2009/30/CE limits to 7% (v/v) the amount of biodiesel to be incorporated into diesel.
In Portugal, the specifications of commercial diesel are established by the Decree-law Nº 214-E/2015. In terms of FAME content, the target of 7% should be evaluated by Infrared Spectrometry – FTIR - according the EN 14078:2014.
At LBA, several diesel samples were analyzed in terms of FAME content using the referred technique as well as gas chromatography. Concerning FTIR analysis, the repeatability of the method was checked by the analysis of an independent standard solution and duplicate analysis. The trueness of results was assured by using a calibration solution prepared from a FAME standard characterized in an International Proficiency Ring Test. The use of this standard also assured the metrological traceability of the results. In this work we present some quality control results of the regression analysis namely the standard deviation of the slope and the intercept of the calibrations functions obtained prior to the analysis of samples.
As certain FAMEs based on coconut oil are coming to the European market, the content of FAME determined by Infrared Spectrometry can be overestimated. Regarding this, our laboratory also analyzed the FAME contents of several samples by gas chromatography with flame ionization detector, GC-FID. It was found a good correlation between the results obtained by FTIR and GC-FID.

P-20

VALIDATION OF LIQUID CHROMATOGRAPHY-QqQ-MS METHOD FOR SIMULTANEOUS QUANTIFICATION OF TWO ANTIRETROVIRALS IN ANIMAL TISSUES AND FLUIDS

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Topical microbicide products combining antiretroviral drugs (ARV) such as tenofovir (TFV) and efavirenz (EFV) are being actively developed for preventing the sexual transmission of HIV-1. Therefore, the development of analytical methods that allow the simultaneous monitoring of several ARV is desirable and recommended. Envisaging the future application to pharmacokinetic studies in mice after intravaginal administration, the major aim of the present work was to develop and validate a ultra performance liquid chromatography-tandem mass spectrometry (UPLC-QqQ-MS) method for quantification of TFV and EFV in biological matrices (mouse vaginal tissue, vaginal lavage and blood plasma).

Chromatographic separation was achieved using a reversed phase Mediterranea sea C18 column (3 μm, 100 x 2.1 mm), maintained at 45 ºC. Elution was performed in gradient mode with 0.1% (v/v) formic acid in water and 0.1% (v/v) formic acid in acetonitrile, at a flow rate of 0.35 mL min^{-1}. Run time was 9 min, with a retention time of 2.8 and 4.1 min for TFV and EFV, respectively. The MS was operated in positive ionization mode (ESI+) for TFV and in negative ionization mode (ESI-) for EFV detection. Data were acquired in multiple reaction monitoring (MRM) mode (TFV, *m/z* 288>176; EFV, *m/z* 314>69). Deuterated ARV were employed as internal standards.

The developed LC-MS method was validated according to EMA guideline on bioanalytical method validation. Calibration curves were linear for ARV concentrations ranging from 4 to 500 μg L^{-1}. The LOD and LOQ for both analytes were ≤0.4 and ≤0.7 μg L^{-1}, respectively. The method proved to be specific and selective. Moreover, precision and accuracy evaluation revealed that intra-day and inter-day variations were within 15%. Mean recovery values of ARV spiked in mice tissues or fluids were consistent and >85%. Matrix effects and stability were also assessed. The proposed methodology was successfully applied to a pharmacokinetic study of vaginal TFV/EFV-loaded nanoparticles in mice.

Acknowledgements: This work received financial support from the European Union (FEDER funds) and National Funds (FCT/MEC, Fundação para a Ciência e a Tecnologia and Ministério da Educação e Ciência) under the Partnership Agreement PT2020 UID/MULTI/04378/2013 - POCI/01/0145/FEDER/007728 and VIH/SAU/0021/2011. L. Barreiros and J. das Neves thank FCT and POPH (Programa Operacional Potencial Humano) for their Post-Doc grants (SFRH/BPD/89668/2012 and SFRH/BPD/92934/2013).

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MICROPLATE-BASED ORAC-PYRANINE ASSAY FOR STRUCTURE-ACTIVITY STUDIES

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Oxygen radical absorbance capacity (ORAC) methodologies measure the protection afforded against oxidation of a target molecule by oxygen radical species, namely peroxyl, by an antioxidant compound. Recently, spectrophotometric target molecules, namely pyrogallol red (PGR) and pyranine (PYR), have been pointed out as alternatives to the classical fluorescent probe fluorescein. However, different target molecules render ORAC values that translate different information due to kinetic differences upon peroxyl scavenging. PGR presents higher reactivity towards peroxyl radical than PYR and, for that reason, ORAC-PGR values reflect the reaction rate between a given free radical and a specific antioxidant (reactivity). ORAC-PYR values, on the other hand, account for the amount of free radicals scavenged by a substance or sample (capacity) because pyranine is less reactive against peroxyl radicals when compared to the majority of antioxidants. An ORAC-PGR microplate routine protocol for antioxidant reactivity estimation has been established by Ortiz et al [1]. As for PYR, the lack of standardized procedures hampers the comparison of data among different studies.

Hence, a high-throughput microplate-based ORAC method employing PYR as target molecule/probe is proposed. To select the best experimental conditions, ORAC values for Trolox (model antioxidant compound) were assessed using 10 and 25 μM of PYR and 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH) for peroxyl radical generation, with concentration ranging from 25 to 100 mM. Plots of lag phase time (min) versus [Trolox] (μM were established. The highest sensitivity (.5.54 ± 0.09 min μM^{-1}) was achieved with 10 mM AAPH, for both PYR concentrations (pseudo-zero order conditions for pyranine). Detection limit was 0.30 μM (Trolox equivalents) and RSD values were < 5.4%. Time interval for data acquisition was set to 120 min. The applicability of the double-target molecule approach was demonstrated by conducting a structure-antioxidant activity relationship study of model biological and food antioxidants, under microplate format. The effect of several properties such as the number of OH groups and their position within phenolic rings, the presence of conjugated double bonds in side-chains and *o*-methylation was evaluated for both ORAC-PGR and ORAC-PYR values.

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P-22

COMBINING WAVELENGTH DISPERSIVE X-RAY FLUORESCENCE AND ATOMIC ABSORPTION SPECTROMETRY TO EVALUATE TOXIC TRACE METALS IN IMITATION JEWELLERY

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Nickel is the most common cause of allergic contact dermatitis and Cadmium is a known human carcinogen and now classified as priority hazardous substances by EUWFD[2] whose emissions to the environment must cease. Thus, the presence of Nickel and Cadmium in imitation jewellery articles is an increasing matter for concern in the European Union and ~~has~~ its use has been restricted under the European Regulation REACH[3]. The maximum Cadmium content allowed is 0.01% (100 mg/kg) by weight of metal (Regulation 494/2011) whereas for Nickel (Regulation 1907/2006; Annex XVII, entry 27) the limit of Nickel release for a period of at least two years of normal use is 0.5 μgNi/cm^2/week.
For Nickel quantification, the European standard EN 1811:2011+A1:2015 shall be used and follows flame atomic absorption spectrometry (FAAS). This technique is a reliable technique to determine trace elements without spectral interferences, but decomposition procedures are required according to matrix and analyte.
Wavelength Dispersive X-Ray Fluorescence (WD-XRF) is an easy and fast technique uses only a small amount of sample and is a non-destructive methodology. Thus, the samples remain available for further analysis by FAAS.
In this study, several imitation jewellery articles collected in the market were qualitatively analysed by WD-XRF in order to not only select the samples which contain Nickel and Cadmium but also to identify the metal alloys in those selected samples. The total amount of Nickel and Cadmium were determined by FAAS, after specific digestion with the samples being digested according with the knowledge of the alloy type of each sample.
For FAAS, global precision under within-laboratory reproducibility conditions was estimated combining duplicate analysis and quality control standard solutions. The trueness component for Ni was determined as recovery from CRMs: low carbon ferro-chromium BCS nº 203/3 and bronze BCS nº 183/1, while for Cd spiked samples were used. The uncertainty of the results was estimated using an intralaboratory relative approach based on the quality control data.
In WD-XRF qualitative analysis the software SuperQ from PANalytical was used to identify which elements were present in the samples, by referring to a database
The combination of these two techniques within the same Laboratory has proven to be a great added value.

[2] EUWFD - European Water Framework Directive (2013)
[3] REACH - Registration, Evaluation, Authorization & Restriction of Chemicals (2006)

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ANALYSIS OF LIQUEFIED SAWDUST USING FTIR AND CHEMOMETRICS

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The use of mid-infrared spectroscopy in combination with multivariate data analysis for the characterization of the solid and liquid phases obtained in pine sawdust's direct liquefaction is presented. Direct liquefaction by solvolysis is carried out at moderated temperatures (100-250ºC) and at atmospheric pressure. In this work, liquefaction experiments were carried out at atmospheric pressure in 100 mL reactor to study the effect on the liquefaction's yield of different variables such as the type and amount of solvent/s, type of catalyst, biomass pre-treatment, reaction temperature and time. After the reaction, the liquid fraction was separated from the solid residue by filtration. The residues were washed with acetone and methanol, dried and weighed to calculate the conversion. The mid-infrared spectra of the solid and liquid fractions were acquired using an ABB BOMEM FTLA2000-100 spectrometer equipped with a DTGS detector, a SiC light source and an ATR sampling accessory (from PIKE Technologies) with a 2 mm wide ZnSe crystal. To extract the maximum information from the FTIR spectra, principal components analysis (PCA) was carried out using Matlab version 7.11 (MathWorks, Natick, MA) and the PLS Toolbox version 4.0 (Eigenvector Research Inc - USA) for Matlab according to the description presented elsewhere [1].

Thus, for example, the effect of reaction time on the spectra of the liquid phases produced using a mixture of diethylene glycol and 2-ethyl-hexanol as solvents and p-toluene sulfonic acid as catalyst is clearly identified using PCA and so 92 % of the variance of the spectra is captured by the first PC. In fact, the increase of the reaction time from 30 min to 2h, which led to the rise of the liquefaction yield from ≈70% to ≈90%, corresponds to an increase of the PC1 scores. Additionally, the spectral regions responsible for the samples' classification can be identified from the loadings plot of each PC. In this case, the loadings plot of PC1 shows that the most significant differences between these spectra appear in the regions: 3200-3500 cm^{-1}, 2850-2950 cm^{-1}, 1720 cm^{-1} and 1100-1200 cm^{-1}. Thus, the changes in the first and last spectral regions are related to the removal of the hydroxyl groups during liquefaction [2]. On the other hand, the peak at 2850-2950 cm^{-1}, which corresponds to the C-H stretching, increases during the reaction. This increase represents the saturation of deoxygenated or cracked sites [3]. Finally, the changes in the peak at 1720 cm^{-1}, associated to carbonyl C=O stretching, are also interesting [3, 4]. In fact, the increase of the carbonyl groups with the reaction time is ascribed to the despolimerization of cellulose with formation of several compounds such as the levulinic acid [3], which is a compound with a high economical value.

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P-24

FLUORESCENT CARBON DOTS FROM CORK INDUSTRY WASTEWATER AS SENSORS FOR PROTEINS

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Carbon dots (C-dots) are quasi-spheric carbon nanomaterials (CNM) with particle sizes of less than 10 nm [1] which are finding increasing applications in fluorescent bio-imaging and nanomedicine [2], sensory analysis [3] and as photocatalysts [4]. Top-down and bottom-up approaches are both being used for their synthesis [1]. Bottom-up processes comprising the treatment of carbon-based industrial waste materials from several forest and vegetable sources for producing CNM are particularly appealing since reduced environmental impacts and high-valued materials may be obtained, paving the way toward bio-based economies.

Within this framework, we report herein for the first time luminescent C-dots obtained from the cork industrial processing wastewater [5] that are able to directly detect cytochrome *c* (cyt *c*) in aqueous medium (pH = 7.2). Cyt *c* plays crucial roles as electron carrier in mitochondrial respiratory system of living organisms as well as in apoptosis [6]. Some of the as-prepared C-dots show quantum yields of 8.1% (λ_{exc} = 380 nm, λ_{em} = 459 nm) and displayed excitation-dependent emission. Given that the fluorescence emission spectrum of C-dots substantially overlaps the absorption spectrum of cyt *c*, a good chance for a Förster-type resonance energy transfer (FRET) between cyt *c* and the protein was anticipated. As expected, cyt *c* is able to quench the C-dots fluorescence (Fig. 1). The sensing ability of C-dots was assessed by the Stern-Volmer formalism; a K_{SV} of 8.5 x 10^{-4} M^{-1} was retrieved from the data.

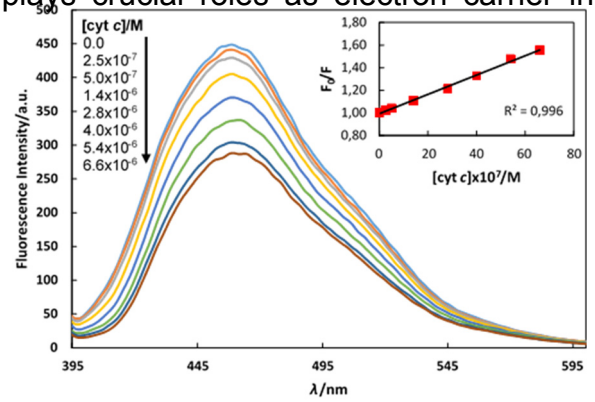

Figure 1: Emission spectra of C-dots (20 μgmL^{-1}) in the presence of increasing amounts of cyt *c* at 25ºC in 50 mM phosphate buffer (pH = 7.2); inset: change in fluorescence intensity ratio at 459 nm as a function of cyt *c* concentration (λ_{exc} = 380 nm).

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P-25

PREPARATION AND OPTIMIZATION OF THIN FLAT POLYSUFONE MEMBRANE FOR BIOMOLECULES PERMEABILITY

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Polysulfone (PSf) membranes are used in different separative processes to broad spectrum of molecules. Nowadays one of the main PSf membranes applications is hemodialysis (HD). The advantage of using this polymer for blood purification is the higher biocompatibility and the greater ability to remove a wide range of uremic toxins, in comparison with formerly used cellulose based membranes. However its clearance ability for middle size uremic toxins, such as β2-microglobulin is not sufficient. Due to the natural hydrophobic character, the PSf membrane is prone to protein fouling on its surface, which may significantly influence the membrane removal efficiency. Moreover, the molecular removal efficiency is also crucially dependent on the membrane porosity, pore size and the pores distribution [1]. To control the membrane characteristics, hydrophilic polymers can be added to the PSf dope solution [2].

In the present work, the effect of additive polyethylene glycol (PEG-6000, 20000, 35000 Da) on the flat sheet PSf membrane solutes removal characteristics was evaluated, as well as the impact of the different humidity conditions on the membrane structure. The thin flat sheet membrane was obtained by phase inversion technique, in which the dope solution was composed of PSf (MW 35000 Da), N-methylene-2-pyrrolidone as solvent and PEG with three different molecular weights as additive. The membrane was prepared by using a spin coater and was conditioned in controlled humidity environments. Deionised water was used as coagulation bath.

The evaluation of membrane solutes removal was performed by permeation study using urea (MW 60 Da), lysozyme (MW~14,3 kDa) and bovine serum albumin (MW~66 kDa). To mimic the conditions of HD, the mini-module of two circuits separated by PSf membrane was connected to a peristaltic pump. The membrane prepared with PEG (20000) shows better results concerning to reproducibility when compared with PEG (35000) and had better permeability adjustment for HD use. The environment humidity conditions had significant effect on pore formation leading to different biomolecules permeability of the PSf membrane. Preliminary results showed that the angular velocity during the membrane deposition has tremendous effect on biomolecules permeability through the membrane.

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OPTIMISATION OF METHYLENE BLUE DEPLETION QUANTIFICATION IN PHOTODEGRADATION STUDIES

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The photodegradation of organic contaminants present in wastewaters not removable by conventional wastewaters treatments, such as medicines active substances and personal care products preservatives, is a promising technology to reduce the impact of these emissions. Nevertheless, catalysts need to be developed to make this technology feasible. Catalysts performance can be assessed from tests in specific contaminants or photodegradation markers such as methylene blue which can be easily quantified by molecular spectroscopy.

The comparison of different catalytic alternatives involves comparing kinetic rates values affected by analytical operations and calibrators values uncertainties. The difference between kinetic rates is meaningful if its absolute value is larger than the expanded uncertainty of the difference which results from the combination of relevant measurement uncertainty components, namely methylene blue concentration before and after irradiation and irradiation time. If the same stock solution is used to prepare both the solution subjected to irradiation and the calibrators for the determination of solution after irradiation, the correlation between relevant uncertainty components must be considered.

This work presents the detailed validation of methylene blue quantification in aqueous solution by molecular spectroscopy and algorithms for estimating methylene blue depletion rates difference. The uncertainty of methylene blue concentration and depletion rates differences are estimated considering relevant variable correlations. These estimations are performed using a user-friendly MS-Excel spreadsheets.

Methylene blue concentration in aqueous solutions is estimated at 246 nm or (550-700) nm, in a range of (0.26-26) mg L^{-1} or (0.26-1.3) mg L^{-1}, with a relative expanded uncertainty between 85%-3.9% or 23%-7.3% respectively. Depletion rates differences are reported with an expanded uncertainty, which represent the smallest distinguishable rate difference, of (0.21-0.044) ms^{-1}. The developed measurement models allow defining optimum quantification conditions and the sound interpretation of kinetic rates differences.

The developed methodology and software can be used in other depletion studies.

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P-27

NANOSTRUCTURED POLYMERIC FILMS FROM DEEP EUTECTIC SOLVENTS FOR ANALYSIS OF PHARMACEUTICAL COMPOUNDS

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Deep eutectic solvents (DES) have recently emerged as innovative solvents in various areas, including nanotechnology, due to their particular properties as new green solvents, offering an inexpensive, biodegradable and robust alternative to conventional ionic liquid solvents [1]. Such eutectic mixtures can also have an active role in tailoring the size and morphology of nanomaterials during chemical or electrochemical synthesis of breakthrough functional nanostructures [2].
By the combination of a quaternary ammonium salt (choline chloride) with a hydrogen bond donor (glycerol), a DES medium was obtained for the synthesis of nanostructured films of poly(methylene blue) (PMB) on glassy carbon electrodes. The electropolymerisation parameters and solution composition were optimized in terms of pH, monomer and water content in order to obtain the best polymer films, which were electrochemically characterized by cyclic voltammetry and electrochemical impedance spectroscopy. An electrochemical quartz crystal microbalance was used to quantify the deposited film and surface characterization by scanning electron microscopy was used to distinguish PMB films of different morphologies depending on the synthesis parameters.
The PMB films obtained under different electrochemical conditions were used for sensing ascorbate, their analytical performance being compared with PMB films obtained in aqueous solution. Applications of the newly developed PMB modified electrodes will be presented, with emphasis on quantification of pharmaceutical formulations.

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P-28

ALKALINE EARTH METALS LEVELS IN THE HUMAN BRAIN TISSUE: ANATOMICAL REGION DIFFERENCES AND AGE-RELATED CHANGES

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Disturbances in brain Ca and Mg homeostasis have been identified as potentially responsible for the cognitive decline associated with normal ageing and development of some neurodegenerative diseases (ND) but the evidence is still fragmentary and its definite role remains unclear. Studies on the levels of Ca, Mg and other alkaline earth metals (AEM) in the human brain are scarce and mostly examine not specified or large brain regions. Since the brain is a highly heterogeneous organ, with anatomically and physiologically very different areas that may be affected in different manners by the aging and neurodegenerative processes, a detailed mapping of AEM distribution across the brain tissue of normal individuals, with an in-depth analysis of AEM levels in the different brain regions, is a prior work, absolutely necessary for interpreting the data obtained from patients suffering from ND and other brain diseases.

Based on this background, our study aimed to contribute to the establishment of robust reference levels of Be, Ca, Mg, Sr and Ba in 14 different human brain regions of “normal” (non-diseased) individuals.

From each neurologically and psychiatrically healthy individual (n=42) the following 14 areas were sampled at autopsy exam: frontal cortex; superior and middle temporal gyri; caudate nucleus, putamen, globus pallidus; cingulated gyrus; hippocampus; inferior parietal lobule; occipital lobe; midbrain; pons; medulla; and cerebellum. After microwave-assisted acid digestion of the samples, Mg and Ca levels were determined by FAAS and Be, Sr and Ba by ICP-MS.

Magnesium (527±34 µg/g) was the most abundant AEM, followed by Ca (226±53 µg/g) and Sr (118±38 ng/g). Barium and Be levels were not detected in any brain region of the studied individuals. Calcium and Sr distribution within brain tissue showed to be quite heterogeneous: the highest levels were found in the occipital and frontal cortex; the lowest Ca levels were found in the medulla and cerebellum while the lowest Sr levels were found in the caudate nucleus and putamen. In specific brain areas, Ca levels seem to be age-related. On the contrary, Mg and Sr seem to remain quite unchanged irrespective of aging.

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NANOHYBRID CARBON DOTS/GSH-CdTe/MPA-CdTe QUANTUM DOTS SYSTEM FOR THE SELECTIVE DETECTION OF IONS IN WATER

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Quantum dots (QDs) and carbon dots (CDs) have been widely used in chemical analysis due to their remarkable optical properties, such as, high quantum yields (QYs), broad absorption profiles and narrow and tunable emission spectra. However, the possible lack of selectivity to a given analyte is probably the main drawback for their use as fluorescent probes. So, this can be compensated by using nanohybrid systems comprising CDs and QDs or a mixture of QDs with distinct sizes and/or cappings and also resorting to multivariate chemometrics methods in order to obtain a pattern-based discrimination of different analytes.

In the present work, CDs and CdTe QDs capped with MPA and GSH, emitting in different wavelengths, were synthesized in aqueous media, which were then conjugated in a nanohybrid CDs/GSH-CdTe/MPA-CdTe QDs system to be used as a nanocomposite fluorometric probe for the detection and differentiation of distinct ions in water.

Taking into account that the nanoparticles involved in the nanohybrid system have different reactivities, the presence of distinct ions triggered different responses of the nanohybrid fluorometric probe. The obtained results were further analyzed through a chemometric method for the detection and quantification of the distinct ions present in the water samples.

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USE OF GAS CHROMATOGRAPHY-MASS SPECTROMETRY FOR DETERMINATION OF MARKERS OF BEER AGING

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Beer stability is a major concern for the brewing industry, as beer characteristics may be subject to significant chemical changes during storage. A variety of flavors may arise, depending on the beer type and the storage conditions [1].

This work aims at evaluating the impact of storage conditions, mainly the temperature and oxygen on beer flavor stability. The profile of some volatile compounds, such as phenylacetaldehyde, phenylethyl acetate and ethylphenyl acetate, responsible for the development of sweet and honey like flavors [2], has been monitored throughout natural (20ºC) and forced aging (37ºC). Beers maintained at 4ºC have been used as controls. The effect of the total oxygen content has also been investigated. The impact of storage temperature and oxygen content on these compounds has been evaluated. The flavor stability of beers has been further evaluated by a well-trained sensory panel, and the sensory data was compared with volatile compounds profile.
Commercial lager beers have been analyzed during an extended storage period (on a monthly basis up to six months), as well as after forced aging (3, 5, 7 and 14 days at 37ºC). Phenylacetaldehyde, phenylethyl acetate and ethylphenyl acetate content have been determined by gas chromatography–mass spectrometry (chromatographic profile and m/z pattern of fragmentation), after extraction with dichloromethane and using 3-octanol as internal standard.

This volatiles compounds has been negatively correlated with the organoleptic quality of beer as evaluated by the sensory panel. Furthermore, phenylethyl acetate has been shown as the better analytical marker of beer ageing induced by heat, among the studied compounds. A 2-fold increase has been observed for phenylethyl acetate content for beers maintained at 37ºC during 7 days, whereas a 4-fold increase has been observed for storage at 37ºC for 14 days.

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P-31

SYNTHESIS AND MODIFICATION OF FLUORESCENT SEMICONDUCTOR NANOCRYSTALS FOR USE IN NON-AQUEOUS MEDIUM

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Colloidal semiconductor nanocrystals, or quantum dots (QDs), have gained increasing interest in recent years because of their advantageous optical and chemical properties, which make their use particularly attractive in different fields.

Aiming to adjust the solubility of aqueous soluble CdTe quantum dots and to enable their usage as fluorescent probes in organic medium, the surface modification of the as-prepared QDs was studied. The modification of the nanoparticles was performed upon binding primary amines with carbon chain lengths varying between C6 and C18, which allowed obtaining QDs with diverse solubility. The binding of the amine to the carboxylic group of mercaptopropionic acid (MPA), used as a stabilizing agent of the CdTe QDs, occurred in the presence of 1-ethyl-3- (3- dimethylaminopropyl) carbodiimide (EDC) and N-hydroxysuccinimide (NHS) that acted as promoters of the amide bond. The optical properties and stability of the obtained modified QDs were characterized to evaluate their applicability.

The modified QDs were tested in the determination of different cardio-selective β-blockers, in pharmaceutical formulations. The best performance was observed for Atenolol which was able to interact with the modified QDs resulting in a decrease of the photoluminescence intensity.

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DEVELOPMENT OF ONLINE AUTOMATED TRANSDERMAL PERMEATION ASSAY APPLIED TO 5-FLUOROURACIL AND SALICYLIC ACID DUAL-LOADED NANOCARRIERS

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One million new cases of skin cancer are diagnosed worldwide every year [1]. Actinic keratosis (AK) is a pre-carcinogenic skin disease requiring effective treatment to prevent its progression to squamous cell carcinomas [2]. Topical formulations combining 5-fluorouracil (5-FU) and salicylic acid (SA) are currently used for AK treatment, avoiding the drawbacks of systemic therapy. However, topical administration is frequently hampered by reduced skin permeation, fostering the development of skin-targeting nanoparticles. In fact, the therapeutic outcome of topical formulations depends on their permeation efficiency, which can be assessed by *in vitro* permeation studies [3].

In the past years, the application of flow-based automated sampling to permeation assays allowed the establishment of kinetic profiles comprising more detailed data as well as the replacement of time-consuming and laborious manual procedures [4]. In this context, a high-performance liquid chromatography (HPLC) method for online monitoring of transdermal permeation of 5-FU and SA dual-loaded nanoparticles is presented. The system comprises a computer-controlled module that performs automated sampling from Franz diffusion cell with withdrawal compensation over time. Real time quantification of analytes permeation is performed by HPLC coupled online to the diffusion cell.

Chromatographic parameters (selection of analytical column, mobile phase composition and flow rate) were established. Linearity between 0.1 - 30 mg L^{-1} was achieved, with a limit of detection < 0.1 mg L^{-1} for both analytes. Application to dual-loaded polymeric nanoparticles and implementation of automated sampling procedure are currently under development.

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FLOW-BASED STRATEGIES FOR THE DETERMINATION OF TOTAL ACIDITY IN WINE

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The acidic compounds present in wines play an important role in keeping the wine microbiologically and chemically stable. These have a great effect on the colour, taste and stability of the final product. As a result, total acidity is an important index of wine character and quality.

The determination of total acidity in wine is a routine procedure described by the *Organisation Internationale de la Vigne et du Vin*. It is defined as the sum of its titratable acidities when titrated to a pH value of 7 against a standard alkaline solution, by potentiometric or visual titration with bromothymol blue (BTB) as indicator. The described method is time consuming, requiring skilled labour. An automation of the analytical methodology can be an interesting alternative to overcome these disadvantages. Flow-based systems have gained increased importance on the automation of the steps required by the official methods for quantification of several analytes.

In this context, two different strategies of flow systems, flow injection analysis (FIA) and sequential injection analysis (SIA), were developed for the automation of this methodology. In the developed methods, the reagent solution was composed by a mixture of OH^- and the pH indicator, BTB. In the presence of the sample, a change on the colour of the carrier solution is achieved and quantified by spectrophotometry. It was possible to establish a linear range up to 0.16 g/L expressed as tartaric acid.

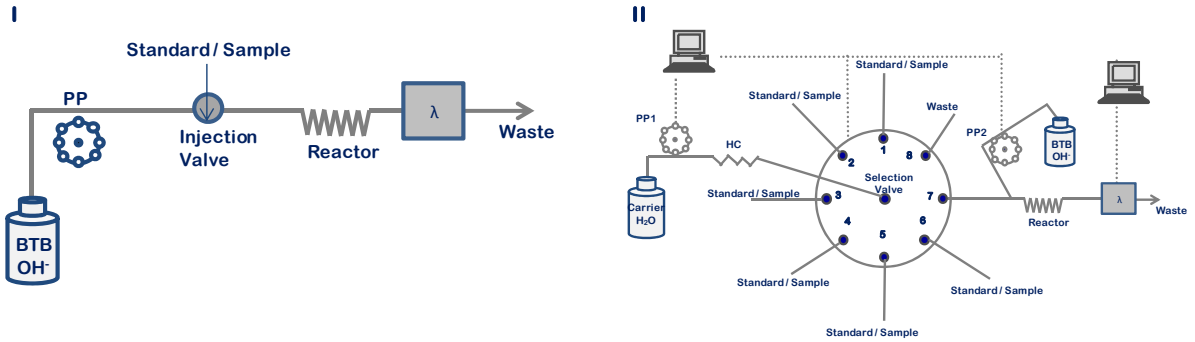

Figure 1: Configuration of the developed manifolds for the quantification of total acidity in wine by flow injection analysis (I) and sequential injection analysis (II).

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A SOLID PHASE EXTRACTION FLOW INJECTION METHODOLOGY WITH SPECTROPHOTOMETRIC DETECTION FOR THE ZINC DETERMINATION IN PLANT DIGESTS

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Zinc plays an important role in plant metabolism; the most significant is its activity as component of various enzymes. However, it is very toxic at high concentrations. Its concentration is related with the chemical composition of the growth media [1]. Zinc is widely used in many industries and this way it is introduced in the environment.
Several methods are available for zinc determination in plants digests, such as Atomic Absorption Spectrometry (AAS) or Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES). These methods present high selectivity and low limits of detection. However, also presents some limitations, such as relatively high equipment cost and consumption of toxic gases [2].
In this work, a flow injection methodology displaying analyte enrichment and spectrophotometric detection for the zinc determination in plant digests is described. The method is based on a solid phase extraction for zinc preconcentration and removal of some interferences, and the colorimetric determination involving Zincon. To implement this approach, an injector commutator and a multi-reflection flow cell were used.
The developed system provides a simple and reliable determination of zinc in plants, with a limit of detection of 0.04 mg/L. When applied to plants digests the results were in agreement with those obtained with reference procedure (AAS).

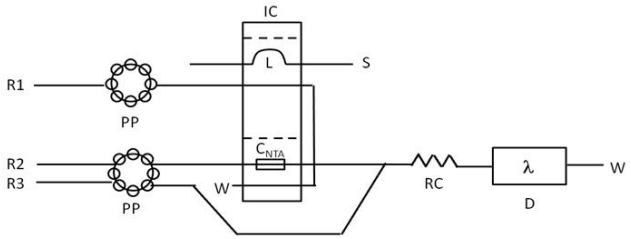

Figure 1: Flow injection manifold for the Zn determination in plants digests. S – Sample/Standard solution; R1 – ultrapure water; R2 – HNO_3, R3 - Zincon; PP – Peristaltic pump; IC – Injector commutator; L – loop; C_{NTA} – NTA column; RC – Reaction coil; D – detector; W - waste

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P-35

INFLUENCE OF GOLD NANOPARTICLE DISPERSION IN MULTI-WALLED CARBON NANOTUBES ON THE ELECTROCHEMICAL DETERMINATION OF THEOPHYLLINE

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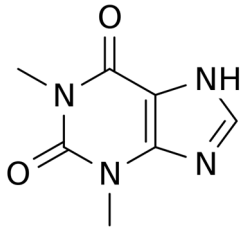
Theophylline (TP) is a commonly used drug for the treatment of respiratory diseases such as asthma due to its bronchodilatory effects. However TP exhibits a narrow safety range, which means that technologies are necessary which have the ability to monitor the levels of TP within the body.



Theophylline

Current laboratory procedures for theophylline detection include radioimmunoassay, high performance liquid chromatography, and fluorescence polarization immunoassay. However, such methods require skilled personnel, sample pre-treatment and a long analysis time [1].

Electrochemical detection is an alternative method which has attracted attention due to its fast response, low-cost instrumentation as well as affordable cost per sample analysis, simple and timesaving operation, high sensitivity and selectivity. In electrochemical analysis, the key component is electrode modification, which requires the selection of suitable materials to improve the analytical performance. To date, many materials have been synthesized and used as electrode modifier materials for TP detection, such as multi-walled carbon nanotubes, graphene, polymers and nanoparticles [2].

In the present work, a new electrode configuration was investigated consisting of gold nanoparticles dispersed in a multi-walled carbon nanotube network deposited on a glassy carbon substrate. Different techniques were used for the characterization of the modified electrode including scanning electron microscopy, UV-Vis spectroscopy, cyclic voltammetry and electrochemical impedance spectroscopy (EIS). The analytical determination of TP was carried out using differential pulse voltammetry and EIS. Parameters that influence theophylline determination were optimized, such as carbon nanotube and gold nanoparticle loading, buffer pH, scan rate and accumulation time and potential. The results achieved were compared with those from the literature with similar electrode architectures. Selectivity, stability and other application will also be discussed.

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ISOTRETINOIN ELECTROANALYTICAL AND SPECTROPHOTOMETRIC DETERMINATION

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Retinoids are naturally occurring compounds and synthetic derivatives of retinol and exhibit similar activity to vitamin A. The 13-cis-retinoic acid (13-cis-RA or isotretinoin) has revolutionized the treatment of severe acne, although it's many adverse effects. In order to know these effects there is an ongoing need for the determination of the 13-cis-RA pharmacokinetics, as well as for the development of methodologies for its detection and quantitation.

The electrochemical oxidation of 13-cis-RA, at a cathodic pre-treated boron doped diamond electrode (BDDE), by cyclic, differential pulse and square wave voltammetry, in a wide pH range, and its UV-Vis spectrophotometric behaviour, were investigated.

The 13-cis-RA oxidation at the BDDE, using cyclic voltammetry, showed an irreversible, diffusion-controlled process that occurred in a three-step cascade mechanism. The differential pulse voltammograms confirmed these processes and showed that in acid electrolytes, the first oxidation reaction involved a proton transfer, whereas the second and third oxidation reactions were pH-independent. For electrolytes above pKa, pH > 5.0, all oxidation steps were found to be pH-independent.

UV-Vis spectra of 13-cis-RA recorded at different pHs showed only one absorption band. Increasing the pH, from acid to neutral and alkaline, a change of λ_{max}, from 399 nm to 345 nm, was observed. At the same time, an absorbance increase, due to the increase of the molar extinction coefficient with the pH increase, occurred.

The electroanalytical determination of 13-cis-RA was carried out, at the BDDE, by differential pulse voltammetry, measuring the first oxidation peak current of 13-cis-RA, and a LOD = 0.49 μM and a LOQ = 1.64 μM, with relative standard deviation (R.S.D.) less than 0.10%, were obtained. In the UV-Vis spectrophotometric determination of 13-cis-RA, at λ_{max} = 345 nm, a LOD = 0.38 μM and a LOQ = 1.25 μM, with R.S.D. less than 0.15%, were obtained.

The quantification of 13-cis-RA in the pharmaceutical formulation Roacutan®, using differential pulse voltammetry and UV-Vis spectroscopy, was performed.

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VALIDATION OF CHROMIUM DETERMINATION IN MARINE SEDIMENTS: COMPARISON OF TOP-DOWN UNCERTAINTY EVALUATIONS

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Marine sediments should be monitored to know the concentration of pollutants in the marine environment in order to assess the health of environmental resources. Heavy metals are pollutants that rapidly reach water systems and are of major concern due to their toxicity, in which chromium is included.
The validation of an analytical method is the metrological treatment from which it is possible to determine if a method complies with the quality required for its use. Therefore the methods should be evaluated as well as tested to confirm that it is suitable for the intended purpose. Thus, the purpose of this work is to validate chromium (Cr) determination in marine sediments. The Cr concentration was determined by microwave digestion of the samples using the OSPAR method or by determining the fraction of metals extracted by empirical method EPA 3050 and the subsequent quantification of the element in the extract by Atomic Absorption Spectroscopy (AAS).
Several parameters have been taken into account and evaluated for the validation of the methods, namely: working range, linearity, sensitivity, detection limits, precision (repeatability and intermediate precision) and trueness.
This work deals with different top-down approaches for calculating the overall uncertainty of the determination of chromium in sediment samples developed by Eurachem and Nordtest. The measurement uncertainty was estimated using results of the analysis of reference materials or laboratory participation in proficiency tests. These approaches are based on the combination of two major uncertainty components, precision uncertainty and trueness uncertainty, which results in the final combined uncertainty.
The obtained results showed that there are not relevant differences in the final uncertainty estimated by studied pragmatic approaches when results of reference materials analysis and OSPAR method is considered. Calculated uncertainties based on proficiency tests results are higher.

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TUTORIAL AND SPREADSHEET FOR THE EVALUATION OF LEAST-SQUARES CALIBRATIONS

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Bottom-up evaluations often underestimated uncertainty in measurements based on instrumental method of analysis, due to the inadequate assessment of calibrators uncertainty component. Calibrators quality must be adequate for the Regression Least-Squares model and their uncertainty has to be taken into account to the sample results uncertainty. The frequently reported correlation between calibrators signals results from the poor quality of calibrators (figure).

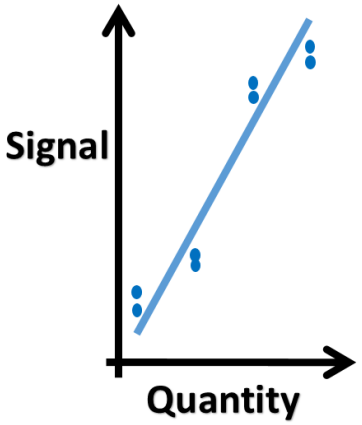

Figure: Correlated calibrators signals

In this work a tutorial for the design, validation and quality control of analytical calibrations is presented [1]. Implemented in a user-friendly and validated MS-Excel spreadsheet, it includes the definition of the calibration range and calibrators preparation procedure, the assessment of regression model assumptions, the estimation of the limits of detection and quantification, the evaluation of the measurement uncertainty and calibration quality control.
The tutorial was applied to the quantification of nitrites in drinking water by molecular spectroscopy, in the range of 0.1 mg L^{-1} to 0.4 mg L^{-1}, with an expanded relative uncertainty ranging from 2.1 % to 3.1 %. Measurement uncertainty estimation quality was checked through the analysis of four control standards distributed along the calibration range, in ten independent calibrations performed in different days. The metrological compatibility of estimated and reference values of control standards proved the adequacy of the measurement model.

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