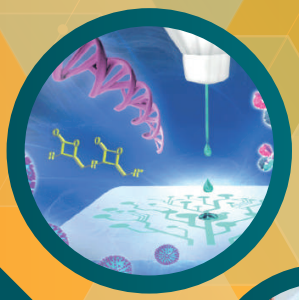
BIOSENSORS

Eleventh International Workshop

03 – 05 October 2024 / Marrakech, Morroco

BOOK OF ABSTRACTS



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08:30 - 18:00

Marrakech, Morroco

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Plenary Conferences

PL1:
WEARABLE CHEMICAL SENSORS AND BIOSENSORS FOR NON-INVASIVE ON-BODY MONITORING

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Non-invasive on-body chemical sensing enables continuous tracking of biomarkers that are central to human health and wellbeing. Information about various analytes can be obtained in a non-invasive manner by chemical sensors and biosensors that are attached to the human skin. Electrochemical and optical transduction methods are most commonly used. Typical approaches include the use of solid-contact ion-selective electrodes for determination of electrolytes (Na^+, K^+, Ca^{2+}, Cl^-) and pH, as well as enzyme-based amperometric biosensors for glucose and lactate [1]. Current research in non-invasive chemical sensing is much focused on the analysis of sweat that is an easily accessible sample as it is naturally excreted from the human body, especially during physical exercise [1]. Among other sample types, saliva and tears receive relatively less attention. Significant efforts are devoted to determination of glucose in interstitial fluid (ISF). Commercially available wearable devices for continuous glucose monitoring mostly rely on biosensors that are inserted through the skin or implanted under the skin to access ISF. This is still not optimal from the users' perspective and a fully non-invasive approach would be preferable. Despite the excellent barrier properties of human skin, non-invasive extraction of ISF is possible without any physical puncture of the skin by utilizing reverse iontophoresis. Furthermore, a recently developed magneto-hydrodynamic (MHD) sampling method was shown to be 13 times more efficient than classical reverse iontophoresis [2, 3]. A wearable and non-invasive glucose monitor based on MHD technology yielded a strong correlation to reference blood glucose measurements in a clinical performance study including over 100 adult participants providing over 900 data points covering a glucose concentration range of 4-26 mM.
In this presentation, a brief overview of non-invasive on-body chemical sensing and biosensing will be given, followed by a specific example of non-invasive glucose monitoring based on MHD extraction of ISF.

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PL2:

ADVANCING SUSTAINABLE NANOBIOSENSORS FOR DIAGNOSTIC APPLICATIONS: INNOVATIONS AND IMPLICATIONS

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The global healthcare system and environmental management face significant challenges due to factors such as an aging population, chronic diseases, rising healthcare costs, pandemics, and environmental pollution. Traditional diagnostic tools, while valuable for their sensitivity and comprehensive capabilities, are often hindered by high costs, complexity, and the need for specialized training and facilities. In response, Point-of-Care (POC) devices offer a promising alternative, providing rapid, accessible, and cost-effective solutions for both healthcare and environmental monitoring. The development of POC devices represents a crucial area of research, particularly in the realms of health diagnostics, environmental control, and food safety.

To meet the growing demand for innovative, sustainable, and affordable diagnostic tools, there is a strong emphasis on leveraging nanomaterials and advanced fabrication technologies. These new devices must embody the REASSURED criteria: Real-time connectivity, Ease of specimen collection, Affordability, Sensitivity, Specificity, User-friendliness, Rapidness, Robustness, Equipment-free operation, and Accessibility. Key questions in this field include: How can we design simple, cost-effective biosensor architectures using materials such as plastics and paper, and enhance their performance through techniques like printing or stamping? How can nanomaterials be effectively integrated with these substrates, and what advantages does this integration offer? Additionally, how can these technologies be linked to mobile communication platforms to extend their utility?

This presentation will address these questions through a series of examples highlighting innovative solutions for detecting critical biomarkers (e.g., cancer cells, viruses) and hazardous environmental compounds (e.g., heavy metals, pesticides). The insights gained from these examples are expected to contribute significantly to the democratization of diagnostic technologies, enhancing healthcare accessibility, and improving environmental monitoring capabilities.

PL3:

MOLECULARLY IMPRINTED POLYMER NANOGELS: SYNTHETIC PEPTIDE ANTIBODIES FOR BIOMEDICAL DIAGNOSTICS AND THERAPY

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Molecularly imprinted polymers (MIPs) [1] are synthetic antibodies that specifically recognize molecular targets. They are cross-linked polymers synthesized in the presence of a molecular template, which induces three-dimensional binding sites in the polymer that are complementary to the template in size, shape and chemical functionality. MIPs against proteins are obtained through a rational approach starting with in silico epitope design. Chemically synthesized peptide epitopes can then be used as templates in a solid-phase protocol for MIP synthesis [2,3].

We demonstrate the potential of MIP nanogels (~50 nm) for diagnostics, bioimaging and medical therapy, on the example of cell surface protein targets [4], as well as soluble cytokines [5].

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Key-note Lectures

KN 01:
A 3D-PRINTED AUTOMATED SAMPLE STORAGE UNIT FOR SPORADIC SAMPLING IN INACCESSIBLE AQUATIC ENVIRONMENT

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Sewage epidemiology has been proven to be a powerful tool to profile a community's behaviour both in large and small areas. Conventional wastewater analysis is based on manually taken samples, subsequent transport to a specialized lab environment and analysis within a certain period of time. However, due to the high logistic efforts, sampling intervals are usually rather long and can hardly be carried out spontaneously or out of a well planned sampling campaign. Therefore, automated sampling devices are becoming popular nowadays, as they can be placed on-site in a single operation and be in stand-by mode during long periods of times waiting to be triggered by a predefined sampling protocol. In this context, we report on a miniaturized, low-cost, easy-to-operate and low power consumption microfluidic automated sampler for sporadic sample collection. The device uses a piezoelectric micropump and three miniaturized electro-valves that are assembled in a 3D-printed microfluidic manifold. Up to three samples can be stored in a 3D-printed single manifold that contains three 2.3 mL reservoirs connected to main body of the device. Moreover, the automated sampler can be remote controlled using a customized control board that enables to trigger the system and set a desired flow rate and time of sampling. Furthermore, its low power-consumption feature enables the device to be powered through a lithium battery. All these qualities make the automated sample device to be very useful for applications where one or several sporadic samples must be taken in poor accessible environments such as the sewer network without the need of personal presence during the sampling event.

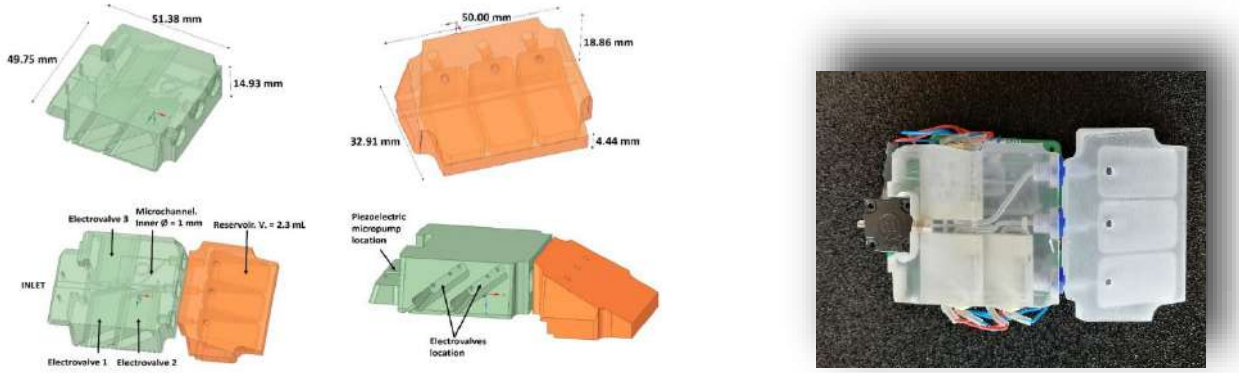

Figure: CAD 3D of the microfluidic manifold and Image of transparent manifolds manufactured using the Project MJP 3600 Max 3D printer (stereolithography).

KN 02:
NANOZYMES FOR NEXT-GENERATION DIAGNOSTIC AND THERAPEUTIC APPLICATIONS

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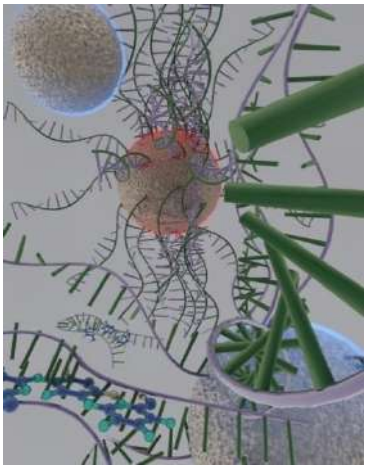
Nanomaterials are well-known for their impressive catalytic activity. However, recently, an increasing number of inorganic nanomaterials have been discovered to behave similarly to the natural biomolecular enzymes, and they are typically referred to as 'NanoZymes'. This biomimetic activity of nanomaterials is establishing NanoZymes as artificial inorganic enzymes, and the research field has just begun to explore this unique property of nanomaterials for a range of applications from diagnostics to therapy. In particular, the focus of our team has been to (i) discover nanozyme activity in new materials (1-3); (ii) develop material synthesis approaches that show multiple or new enzyme-like activities in a single or hybrid material, e.g. nanozymes with apparent mimicry of diverse mammalian enzymes, including unique pan-glycosidases (3); (iii) understand the mechanism of nanozyme activity in nanomaterials (3); (iv) modulate nanozyme activity through external stimuli such as light (4); (v) use nanozymes to develop highly specific colorimetric biosensors (1-2, 5-7) and (vi) explore the potential of nanozymes for therapeutic applications such as pro-drug therapy (3) and photo-activatable antimicrobials (4). We have achieved this progress by studying the nanozyme activity of over 100 nanomaterials which has allowed us to draw some generalized trends about the behavior of nanozymes. In this talk, we will discuss some of these recent developments made by our team.



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KN 03:

SOLUTIONS AND STRATEGIES FOR SMALL MOLECULE SENSING AND ENVIRONMENTAL MONITORING

*Yiran Luo, Tara Barwa, Vanessa Mokwebo, Tony O'Hara, Simon Egan, Baljit Singh, Carmen Negredo, Eoghain Murphy, Saurav K. Guin, Eithne Dempsey**

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2D nanosheets and supported 3D metallic nanomaterials are employed for rapid and sensitive pharmaceutical residue electroanalysis in pharmaceutical and environmental samples. Such refractory chemical species can contribute to antibiotic and antiviral resistance due to their presence in the aquatic system. The methodology proposed employed top-down exfoliation of bulk transition metal dichalcogenides (molybdenum disulfide MoS_2) as compared with an electrodeposited approach for quantitation of the antibiotic metronidazole via a reduction process. The use of a triple pulse electrosynthetic approach for deposition of copper particles on glassy carbon and oxygenated carbon nanoonions is also presented to realise antiretroviral efavirenz electroanalysis and establish an understanding of redox behaviour. The approach was extended to waste-water effluent and extraction of a commercial antiviral solid dose formulation. Given the widespread commercial usage of engineered nanoparticles, an understanding of their cytotoxicity is crucial in managing long term impacts and environmental degradation. This is currently poorly understood, and the use of optical *in vitro* assays based on mitochondrial activity remain the state of the art with challenges of cost and optical interference of agglomerated particles in cell media. An electrochemical assay based on acid phosphatase activity was developed and transferred to a portable *in house* designed and fabricated biochip which enabled cell culture (mammalian and fish cells), fluidic delivery and detection of redox molecules at screen printed transducers. IC_{50} values were estimated for model toxins including quantum dots. Finally, a bespoke enzyme assay for *E* coli bacteria measurement in water was developed with the aid of cell capture membranes, delivery channels and on board reagent cavities achieving low levels of coliform measurement in water samples.

KN 04:
KESTERITE-FUNCTIONALISED ELECTROCHEMICAL APTASENSORS FOR DISEASE BIOMARKERS TOWARD SUSTAINABLE DIAGNOSTIC SENSING

Emmanuel Iwuoha, Onyinyechi Uhuo, Kaylin Januarie, Marlon Oranzie, Jaymi January

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Cape Town, South Africa

Affordable disease diagnostic tests are *sine qua non* for the realisation of the universal good health anticipated by Goal 3 of the UN Sustainable Development Agenda (SDA) 2030. Highlighted in this presentation are the nanoarchitectonic and electro-energetic properties of functional materials and their application in the development of diagnostic sensor devices. Focus would be on biosensors for particular diseases that are prevalent in Sub-Saharan Africa, such as tuberculosis (TB) and myocardial infarction (MI) – a type of heart attack. Accordingly, ampero-impedance phase angle aptasensors for interferon-gamma (IFN-λ), a TB disease biomarker, were developed with biocompatible kesterite- and perovskite-amplified nanomaterials. The presentation will also include our recent work on aptasensors for B-type natriuretic peptide (BNP) and C-reactive protein (CRP), which are two emerging disease biomarkers that indicate high probability of MI.

KN 05:
PHAGE-BASED ULTRASENSITIVE DETECTION OF BIOMARKERS

Chuanbin Mao

Department of Biomedical Engineering, The Chinese University of Hong Kong

Bacteriophages, also called phages, are human-safe non-toxic viruses that specifically infect bacteria. They are one of the most common and diverse entities in biospheres. They can be pictured as nanobiomaterials assembled from proteins and nucleic acids. For example, filamentous phage (such as M13 phage) is a nanofiber (about 1 mm long and 7 nm wide) with multiple genetically modifiable proteins constituting a capsid and a DNA as a core inside the capsid. Since the DNA inside the phages encodes the proteins on the capsid, phages can be genetically engineered to bear the capability of targeting nanoparticles, proteins, cells, tissues, and organs. Therefore, they are ideal for many applications in precision nanomedicine and regenerative medicine. This talk will summarize my group's recent studies on the use of phages for ultrasensitive biomarker detection for disease diagnosis. Specifically, I will talk about the use of phages for detecting miRNAs, antibodies, and circulating tumor cells (CTCs) with high sensitivity and specificity. These studies show that phages are genetically modifiable nanobiomaterials that can be applied as probes to achieve early diagnosis.

KN 06:
CHALLENGES AND OPPORTUNITIES TO REALIZE BIOSENSROS FOR IN VIVO CONTINUOUS MONITORING OF PEPTIDES AND PROTEINS

Koji Sode

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The most prominent example of biosensors today are the sensors used in continuous glucose monitors (CGMs) for diabetes management[1]. CGM systems use a small biosensor inserted or implanted under the skin to measure interstitial fluid (ISF) glucose concentrations every few minutes. The measured ISF glucose concentrations are then converted to blood glucose values, the data is sent wirelessly to a device, and patients receive information about how their glucose levels are changing over hours to days. The advent of CGMs also enabled the development of an automated insulin delivery system in which a subcutaneous pump communicates with a CGM and injects insulin in proportion to both real-time and predicted glucose levels. With the incredible success in realizing the closed-loop system in the use of biosensors to improve therapeutic outcomes for patients with diabetes over the past decade, the continuous sensing of other biomarkers, such as peptides and proteins, is of emerging interest to both researchers and clinicians.

A biosensor, according to its definition, consists of at least one biological recognition element that specifically recognizes the target and generates the signal to a transducer[2,3]. A biological recognition element is then categorized into two types; a biocatalytic recognition element and a bioaffinity recognition element. Glucose and other small organic molecules can be detected based on several biocatalytic recognition elements represented by oxidoreductases. The catalytic sites of these biocatalytic recognition elements, which are continuously regenerated after recognition, provide constant signals that can be detected by transducers, which are suitable for constituting sensors for continuous monitoring systems.

For the detection of protein/peptide biomarkers in picomolar (pM) to nanomolar (nM) concentrations, current biosensor principles use bioaffinity recognition elements such as antibodies and aptamers. This is due to their high binding constants (K_D=10^{-8} to 10^{-10} M). The advantages of using such highly sensitive and selective bioaffinity recognition elements are at the same time the disadvantages in using them to create a continuous monitoring system. Namely, the target analyte molecules bind so tightly to the bioaffinity recognition elements that regeneration of these biosensing elements is only possible under harsh chemical conditions or by increasing temperature. Therefore, the most challenging task to realize biosensors for continuous in vivo monitoring of peptides/proteins using bioaffinity recognition elements is the in situ regeneration of biosensors[4].

In this talk, I will present the challenges and opportunities to realize biosensors for in vivo continuous monitoring of peptides/proteins by developing engineered bioaffinity recognition elements.

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KN 07:
TRANSDERMAL DIAGNOSTICS BASED ON MICRONEEDLE PATCHES

Peng Chen
Nanyang Technological University (Singapore)

Skin offers an easily accessible window for minimal invasive detection of various biomarkers in the skin interstitial fluid (ISF). Formed by capillary filtration, skin ISF shares the common presence (>90%) of biomarkers as in plasma. And the variation dynamics of the small analytes in ISF is similar to that in plasma. Transdermal diagnosis based on microneedle patches integrated with various sensing elements promises for personalized, home-based, and long-term monitoring of chronic diseases, due to its minimal invasiveness, convenient administration, good patient compliance. In this talk, I will share our recent works on multiplexied transdermal detection of metabolites, ions and chemicals that are associated with various diseases. For example, we recently developed a microneedle coupled epidermal sensor for multiplexed electrochemical detection of kidney disease biomarkers. In another example, we developed colorimetric microneedle patches for multiplexed transdermal detection of metabolites.

KN 08:
ELECTROCHEMICAL PAPER-BASED ELECTROCHEMICAL (BIO)SENSORS FOR THE NEXT GENERATION OF ANALYTICAL TOOLS

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As reported in my recent review entitled "Electrochemical paper-based devices: When the simple replacement of the support to print ecodesigned electrodes radically improves the features of the electrochemical devices" published in Current Opinion in Electrochemistry SI: Emerging Opinions (2022), "Paper-based electrochemical (bio)sensors have emerged as highly attractive analytical devices for their superior sustainable features, such as avoiding the use of polyester as support and the reduction of waste, being incinerated after use. However, paper-based electrochemical (bio)sensors have recently demonstrated further advantages, including the simple combination with vertical microfluidics and their use as a reservoir to deliver smart electrochemical (bio)sensors able to i) contain the reagents, ii) preconcentrate the target analyte, and iii) synthesize the nanomaterials inside the paper network. Furthermore, these devices have demonstrated their ability to overcome the limitations of the other printed electrochemical sensors in the measurement of entirely liquid samples by detecting the target analyte in the aerosol phase or solid sample, without the additional sampling system. These achievements highlight their valuable and varied advantages in the sensing sector". Herein, I report the recent results of my group related to electrochemical paper-based devices in unconventional configurations. We recently demonstrated a hybrid configuration for a market entry device for virus detection in saliva (patent filled), a wearable paper-based immunosensor washing-free for cortisol detection in sweat (Sens. Actuat. B 2023, https://doi.org/10.1016/j.snb.2022.133258), and a paper-based device combined with polyvinyl chloride electrochemical system in which a paper layer loaded with reagents is inserted into this device, working as a new concept of paper card-like for a reagent-free and easy measurement of target analyte (i.e. glucose) in solution (i.e. tears) (Chem. Comm. 2023, https://doi.org/10.1039/D2CC06561D, selected for the cover page). Thanks to the advanced features of paper-based electrochemical devices, we are now working on innovative paper-based electrochemical (bio)sensors embedded in a paper-based origami organ-on-a-chip within the European Pathfinder Open Phoenix-OoC project.

Oral Communications

CO 01:
LASER PLOTTER-BASED STRATEGIES FOR BIOSENSORS AND PAPER-BASED ELECTROANALYTICAL DEVICE DEVELOPMENT

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Nanomaterials synthesis and integration into biosensors and paper-based analytical devices (PAD) still represent a critical issue. To overcome tedious, expensive, and not sustainable conventional fabrication techniques, several efforts are devoted to implementing effective and affordable technologies to produce nano-equipped devices. Benchtop-scale CO2 laser plotter-based technologies represent a captivating opportunity to produce graphenic and graphitized surfaces/structures and drive nanodecoration/heterostructure formation.
This presentation will be focused on the production of different functional nanostructured films via CO2-laser plotter and their integration within completely lab-made biosensors and PAD, with the idea of reducing the use of solvents/chemical compounds as much as possible and generating virtuous circles from an environmental and economic point of view. Particular attention will be paid to biosensors and PAD manufacturing using low-cost/sustainable substrates (i.e., nitrocellulose, paper, recycled paper, paper produced from wastes, etc.) via benchtop microfabrication technologies such as stencil-printing, cutter-plotting, CO2-laser molding, thermal-lamination, etc.
The exploitability of the developed devices will be demonstrated for the analysis of agri-food quality and safety markers, and biological interest compounds in model solutions and real samples. Among others, will be presented: (i) electrochemical sensors based on laser-induced reduced graphene oxide films integrated into recycled papers and paper produced from wastes able to determine different analytes in different samples (food, supplements/medicines, urine), (ii) activated inks/graphene-based 3rd generation biosensors for the determination of fructose and fructose-based compounds in food and biological samples, (iii) an integrated paper/graphene 3D pop-up device for the quantitative sensing of carbaryl in grains at EU-law limits, (iv) additional findings concerning the developments of others electroanalytical biosensors and PAD will be eventually presented.
This presentation aims to prove how CO2-laser plotter-based strategies are able to generate nanostructured functional sensing films, that via benchtop technologies can be integrated within cost-effective, flexible, and sustainable substrates to give rise to everyone-reach effective analytical devices.

CO 02:

METAL NANOPARTICLES LASER-WRITING ON CELLULOSIC SUBSTRATES FOR COLORIMETRIC PAPER-BASED ANALYTICAL DEVICE DEVELOPMENT

A. Scroccarello1, F. Della Pelle1, P. Di Battista1, S. Fiori1, D. Compagnone1.
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In this presentation, a CO2-laser plotter-based versatile strategy to in-situ synthesize on paper plasmonic active gold (Au), silver (Ag), platinum (Pt), copper (Cu), cerium (Ce), nickel (Ni), and aluminium (Al) nanostructures will be presented [1]. This approach allows the formation of metal-nanostructures, named Laser-Induced Metal nanoparticles (LIMs), on different cellulosic substrates including Whatman paper, office paper, and recycled/byproducts-based papers without the need for external reagents.
The laser allows LIMs' design, shaping, and anchoring onto paper in a single stroke in a few seconds with the desired configuration. Noteworthy, LIMs possess features useful for sensing purposes, resulting in plasmonically active, catalytic, and photoluminescent according to their chemistry and morphology. LIMs have been used to equip different lab-made colorimetric paper-based analytical devices (PAD), conceived to work with smartphone-based readouts. The proposed PADs were manufactured using low-cost benchtop technologies (i.e., laser/cutter-plotter, thermal-laminator, etc.) and office-grade substrates (i.e., polymeric and cellulosic substrates). In this presentation, different LIM-based PADs will be presented, including, (i) a three-channel paper fluidic device (LF3) equipped with Ag, Au, and Ce-LIMs for the hypochlorite determination in milk, in the framework of bleaching frauds; (ii) a 'Flip-PAD' for the rapid (1 min) and selective determination of ascorbic acid in food and supplements, in this case, the device working mode relies on the Pt-LIM oxidase-like activity that allows colorimetric dye conversion; (iii) a fluorometric PAD equipped with photoluminescent Al-LIM for the selective quali-quantitative detection of o-diphenols in food samples.
The herein proposed laser writing strategy turns out an innovative and sustainable nanopatterning technique, prone to generate optical sensing zones useful to develop (bio)sensing strategies and manufacture within everyone's reach PAD.

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CO 03:

LASER-PLOTTER MANUFACTURED COLORIMETRIC FOUR-LEAF CLOVER-LIKE MIP/PAD FOR MALEIC HYDRAZIDE DETECTION

D. Elfadil1, A. Scroccarello1, F. Della Pelle1, P. Di Battista1, A. Amine2, D. Compagnone1

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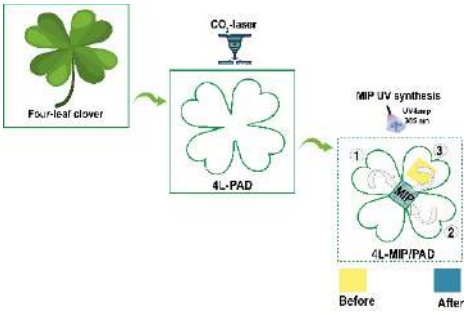
A laser-based approach to manufacture a colorimetric Paper Analytical Device (PAD) integrating Molecularly Imprinted Polymer (MIP) for maleic hydrazide (MH), is proposed. The device has a four-leaf clover-like shape (4L-MIP/PAD) and was desing to integrate the MH complete analysis; MH is an anthropic plant growth regulator widely spread/found in vegetables.

The 4L-MIP/PAD has been shaped with a CO2-laser plotter and integrates a CO2-laser-activated fiberglass layer functionalized with UV in-situ synthesized MIP. The 4L-MIP/PAD graphical sketch is reported in Scheme 1; the PAD houses the MIP at the center, while each foldable leaf allows the implementation of a specific analysis step.

4L-MIP/PAD based MH analysis procedure: a drop of the sample extract (40 µL) is deposited on the MIP, (1) the sample is removed by paper capillarity folding the 'absorption-leaf', the MIP washing is performed depositing a water drop on the MIP, (2) the washing-phase was removed folding the 'washing leaf'. Eventually, the analyte is eluted depositing a drop of an alkaline solution on the MIP, (3) then the folding of the colorimetric left allows the colorimetric reaction; the latter relise, on the on-paper adsorbed Folin-Ciocâlteu reagent. The 4L-MIP/PAD-based complete MH analysis requires 30 min, and the colorimetric signal acquisition needs just a smartphone camera. 4L-MIP/PAD-based ensures the MH determination between 10 to 100 ppm (RSD≤13%, n=3), showing a LOD of 3.3 ppm. These figures of merits allow cover the admitted residual law limits for root and tuber vegetables (maximum residue level: 50 ppm for potatoes, 30 ppm for carrots, and 15 ppm for garlic and onions). The 4L-MIP/PAD selectivity was tested against different kinds of pesticides and several food matrix components (vitamins, amino acids, organic acids); despite the non-selective of the Folin-Ciocâlteu reagent, the 4L-MIP/PAD exhibits a high selectivity toward MH. Tests on the device stability and the application in food samples are actually on course.



Scheme 1. Graphical sketch the Four-Leaf Clover-like MIP/PAD

CO 04:
ELECTROCHEMICAL BIOASSAY FOR DETECTION OF HUMAN PAPILLOMAVIRUSONCOGENIC ACTIVITY

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Human papillomavirus (HPV) is known for being the crucial transforming agent of cervical cancer, but also contributes to oropharyngeal and anogenital cancers progression. Cervical cancer is the most common from HPV-induced cancers and belongs among five leading causes of death from tumor diseases in women worldwide. Higher prevalence is observed in countries lacking preventive programs, comprising especially HPV vaccination and screening.
Nowadays, standard screening methods and commercial tests for HPV detection in clinical samples are mostly based on PCR and are designed to detect viral DNA. This approach allows to detect presence of the virus in cells but does not provide information about the viral oncogene transcriptional activity, which may better reflect progression of the disease (1). Therefore, new methods for detection of HPV mRNAs, and cheaper, easier and faster alternatives to PCR at the same time, are being developed.
After previous works that focused on DNA detection (2, 3, 4), here we present an electrochemical-based assay using isothermal loop-mediated amplification (LAMP), so-called EC-LAMP assay, for detection of the HPV16 mRNA biomarkers in cervical samples of women with precancerous lesions reflecting oncogenic activity of the virus in host cells. In this approach, mRNA levels of three genes were measured: E2 as a regulation gene, E7 as an oncogene and 18S rRNA subunit as a housekeeping standard. Ratios between E2 and E7 mRNA levels were calculated, and results compared to clinical data as well as to standard methods, PCR and ddPCR. The assay showed good concordance with both clinical data and standard validation methods, and could be potentially useful for early HPV diagnostics before cervical cancer develops.

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CO 05:
BIOELECTROCHEMICAL DETECTION OF CANCER BIOMARKERS

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Cancer biomarkers are widely recognized as useful tools for a broad spectrum of oncological diseases, serving in the early stages of diagnostics, molecular characterization when selecting proper treatment, and prediction of therapy response. Cancer biomarker detection is often challenging because of the low levels of these biomarkers in the early phases of the disease. Therefore, higher demands are placed on the overall sensitivity of these methods. Nucleic acid-based cancer biomarkers are a highly heterogeneous group, including DNA biomarkers such as oncoviral DNAs, single nucleotide polymorphisms (SNP), DNA methylation, or chromosomal amplification, as well as RNA biomarkers, including elevated levels of specific mRNAs or altered levels of non-coding RNAs, especially long non coding RNA (lncRNA), and various microRNAs (miRNAs) [1].
Our research combines isothermal amplification techniques (IATs) such as loop-mediated isothermal amplification (LAMP), rolling circle amplification (RCA), or recombinase polymerase amplification (RPA) with electrochemical readout. In our bioassays, we employ IATs in the presence of modified nucleotides to amplify target nucleic acids, which are then hybridized on magnetic beads or electrode surfaces, followed by peroxidase labeling and electrochemical (EC) readout. This combination offers benefits such as low instrumentation, short reaction times, specificity, and a highly sensitive detection approach [2].
We have successfully combined IATs techniques in our work, for example, for the detection of human papillomavirus [3-6] and human cytomegalovirus nucleic acids associated with cancer development and progression, analysis of SNP in the BRAF oncogene [7, 8], and detection of elevated levels of PCA3 lncRNA in prostate cancer [9]. We strongly emphasize the feasibility of our assays in clinical materials, such as cervical swabs, urine, tissue samples, or serum used for liquid biopsy. We believe that EC-based detection methods can be considered interesting and inexpensive tools for cancer biomarker detection in clinical laboratories.

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CO 06:
NANOBODY-BASED DIAGNOSTIC TOOLS FOR TOXIN DETECTION

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Every year, numerous people are hospitalised following contact with natural toxins such as animal venoms, poisonous plants, and bacteria. Currently, the detection of these toxins is restricted to centralised laboratories, typically requiring extensive time, expensive instrumentation, and highly trained specialists. As such, there is an increasing demand for user-friendly tools that can rapidly and reliably detect these toxins outside of central labs. Affordable antibody-based tests such as lateral flow assays (LFAs) could offer a promising solution, but so far, LFAs lack the robustness, sensitivity, and specificity of lab-based analysis. The limitations of LFAs can be partly explained by the way in which test line antibodies are immobilized onto the nitrocellulose membrane. Most often antibodies are attached by passive adsorption but that leads to random fixation of antibodies thereby compromising assay performance. Alternatively, antibodies can be attached to the membrane covalently or via an affinity linkage agent, but both of these methods require modification of the paper-based substrate, manipulation of the immunoreagent, or both. Nanobodies are antibody fragments that offer advantages compared to traditional antibodies due to their smaller size, increased stability, sensitivity, and specificity. Therefore, state-of-the-art nanobody technology could be exploited to develop innovative paper-based diagnostics. Still, immobilizing nanobodies onto nitrocellulose can be problematic given the hypothesis that they are more prone to suboptimal immobilization through passive adsorption compared to traditional antibodies, owing to their smaller size. To overcome these limitation, cellulose-binding modules (CBMs) can be fused to nanobodies to facilitate their direct immobilization onto paper-based substrates. In this work, we have developed an LFA using a recombinant bifunctional protein consisting of a nanobody fused with a CBM which allows the protein to be anchored to cellulose as the capture agent. The LFA with a nanobody-CBM test line was able to detect the target antigen with greater sensitivity (i.e., a lower limit of detection) compared with an LFA with just the nanobody as the test line. Furthermore, employing a CBM enabled the immobilization of nanobodies onto cellulose, something which is typically hindered by a high risk of steric hindrance and protein desorption during washing steps. In contrast to the traditionally used nitrocellulose, cellulose could provide an affordable and (bio)sustainable alternative substrate for micro-paper analytical devices. This research could result in an easy-to-use and versatile diagnostic platform that can be adapted for the sensitive detection of other toxins and contaminants with the potential to shift how envenomations and poisonings are diagnosed at the point of care.

CO 07:

NEUROTOXIC COMPOUNDS SCREENING USING BIOSENSOR WITH CO-IMMOBILIZED ACETYLCHOLINESTERASE AND BUTYRYLCHOLINESTERASE ON ELECTRODES MODIFIED WITH CUNPS AND STABILIZED PRUSSIAN BLUE

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The organophosphorus and carbamate neurotoxic insecticides are analysed with monoenzymatic biosensors usually based on acetylcholinesterase or rarer on butyrylcholinesterase. These monoenzymatic biosensors are not able to detect the entire spectrum of insecticides since there are some compounds that inhibit only one of the two enzymes, e.g. pirimicarb inhibits at butyrylcholinesterase at much lower concentrations than acetylcholinesterase while methomyl inhibits only acetylcholinesterase. We have developed a bienzymatic biosensor that contains both acetylcholinesterase and butyrylcholinesterase co-immobilized on the same electrode and each insecticide is determined by the enzyme with the highest affinity. The electrodes were modified with a stabilized copper containing Prussian blue electrodeposited on electrodes modified with 4-aminothiophenol monolayer using diazonium chemistry and copper nanoparticles for improved sensitivity. Our system is simple and in a single measurement provides a sensitive alarm for insecticides' presence based on the inhibition of the enzyme with the highest affinity for each toxic compound. The limits of detection are 50 ng/mL pirimicarb for bienzymatic biosensor in comparison with 400 ng/mL pirimicarb for acetylcholinesterase biosensor and 6 ng/mL methomyl for bienzymatic biosensor while an inhibition is obtained for butyrylcholinesterase biosensor at 700 ng/mL. The proposed bienzymatic system was also applied for the analysis of natural toxins released during cyanobacterial blooms in stagnant water bodies. Early detection of cyanobacterial toxins by using AChE-based biosensors may thus be a useful approach to prevent potential harmful effects on resident fauna or humans.

CO 08:

A HYDROGEL NANOCOMPOSITE IMMUNOSENSOR FOR THE ELECTROCHEMICAL DETECTION OF HEMOGLOBIN

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Biosensors exploit the incorporation of biological materials into monitoring devices using inorganic/organic materials [1]. However, since bioelectronics based on organic/inorganic materials have rigid mechanical properties and low biocompatibility, it is challenging to use them in real-world biomedical diagnostics [2]. To address these limitations, hydrogels are being explored as flexible, high-swelling polymers capable of adsorbing biological recognition elements in sensing devices associated with microfluidics and robotics [3,4]. Indeed, hydrogels exhibit high biocompatibility due to their properties such as high moisture content, biodegradability, and porous structure. However, hydrogels show limitations, as low structural stability, conductivity, and cell adhesion properties. Therefore, biohybrid hydrogel combined with nanomaterials can overcome these limitations offering the synergistic effect of hydrogel biocompatibility together with the high conductivity of nanomaterials [5]. To this aim, a novel hybrid hydrogel nanocomposite immunosensor was developed for the electrochemical detection of hemoglobin in blood. The transducing element combined carbon black (CB)-nanomodified screen-printed electrodes (SPEs) and a poly(ethylene glycol)-diacrylate hydrogel (PEGDA) doped with gold nanoparticles (AuNPs). Electrochemical Impedance Spectroscopy (EIS) and Differential Pulse Voltammetry (DPV) analysis was performed to follow the fabrication of the PEGDA-AuNPs/CB-SPEs, as well as its oriented functionalization with the anti-hemoglobin antibody. The analytical performances of the resulting PEGDA-AuNPs/CB-SPE immunosensor were assessed by DPV analysis, demonstrating the ability of the proposed system to detect hemoglobin in standard solution and serum samples with a limit of detection of 0.005 mg/mL within a dynamic response in a concentration range from 0.005 to 0.1 mg/mL. Any interference was observed in the presence of glucose and ascorbic acid at concentrations much higher than those physiologically present in the blood. Moreover, any matrix effect was evidenced in serum samples, with a good recovery value of 107 ± 4 % obtained for a hemoglobin concentration of 0.025 mg/mL.

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CO 09:
VERSATILE CHIMERIC PROTEINS FOR IMMUNO-BIOSENSORS DEVELOPMENT

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Filamentous fungi usually produce hydrophobins (HPBs), because of their ability to self-assemble into stable, amphipathic layers, to decrease interfacial tension and to allow the development of aerial structures [1]. Thanks to their ability to adhere to surfaces, these proteins can be a versatile platform for functionalizing materials, alone or combined with other biomolecules. Indeed, protein engineering offers appealing chances to create chimeric proteins by gene fusion. This strategy allows the combination of the adhesive ability of HPBs with the specific properties of different fusion partners, through the strength of covalent peptide binding. Derivatizing surfaces with such biomolecules is a step forward in the biosensing field since it represents a new and sustainable immobilization approach, eliminating time and cost-consuming processes. The HPB Vmh2, extracted from Pleurotus ostreatus, has been fused to different biomolecules to develop immunosensors for the detection of molecules of interest.
In detail, Vmh2 has been fused to Protein A, able to bind the heavy chain within the Fc region of immunoglobulins G (IgGs) without compromising their ability to bind antigens. The fusion protein has been used to functionalize gold nanoparticles as a platform for immobilizing different antibodies. The immunosensor obtained showed an adequate limit of detection (LOD). Other examples are the fusion proteins obtained by Vmh2 and the Single-chain fragment variables (ScFv) against two marine neurotoxins, Saxitoxin and Domoic Acid [2]. In this case, magnetic beads were used as a platform for the fusion proteins, that could detect the toxins at very low concentrations.

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CO 10:
HIGHLY SENSITIVE IMPEDIMETRIC BIOSENSOR FOR ENVIRONMENTAL DNA DETECTION

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Environmental DNA (eDNA) has emerged as a powerful tool for species detection, biomonitoring and surveillance, offering greater sensitivity and efficiency compared to traditional survey methods. [1] Therefore, developing a simple and sensitive detection system for real-time eDNA monitoring is meaningful and highly desired. In this context, a biosensor emerges as an exemplary solution due to its manifold advantages, particularly impedimetric biosensors, which offer elevated sensitivity, selectivity, real-time detection, and cost-effectiveness. For this purpose, we are developing an Electrochemical Impedance Spectroscopy (EIS)- based biosensor for eDNA detection. Previously, we successfully functionalized gold screen-printed electrodes (AuSPEs) with antibodies, achieved through the design of a customized microfluidic cell that enabled the exclusive interaction of various solutions with the working electrode [2]. Currently, we are focusing on optimizing the process of functionalizing AuSPEs with single-stranded DNA (ssDNA) probes. Once functionalized, the ssDNA-modified electrode is exposed to a solution containing the complementary target ssDNA, resulting in hybridization and the formation of double-stranded DNA (dsDNA) on the electrode surface. This interaction is monitored using EIS, and our preliminary results are promising. Specifically, using ssDNA derived from S. cerevisiae, we are able to detect concentrations in the range 1-100 pM, with significant potential for further reducing the detection limit. The specificity of the biosensor was validated using a non-target sequence, which resulted in no detectable signal. Therefore, our proposed biosensor demonstrates high specificity, rapid detection capabilities—achieving results in less than one hour—and suitability for on-site measurements.

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CO 11:
RAPID COLORIMETRIC BIOSENSOR FOR ENVIRONMENTAL DNA DETECTION

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Over the past decade, environmental DNA (eDNA) has emerged as a promising tool for studying biodiversity, utilizing genetic markers to deduce the presence of various species.[1] Currently, eDNA detection is carried out using different approaches based on the use of Polymerase Chain Reaction (PCR). This technique is expensive, time-consuming and requires specialized personnel.[2] In this scenario, it is highly desirable to develop a rapid colorimetric biosensor for the detection of eDNA, which can also be employed for real-time analysis. Our approach is based on the competitive and specific aggregation of gold nanoparticles induced by the absence of the target (eDNA). To this aim, we synthesize two distinct batches of functionalized gold nanoparticles (f-AuNPs), each having a different and complementary single-stranded DNA (ssDNA) sequence. In the absence of the target analyte, mixing the two batches of f-AuNPs together will result in aggregation due to the interaction between their complementary ssDNA sequences. On the contrary, aggregation will not be visible when the target analyte is recognized by one of the two batches of f-AuNPs. This technique has been used to detect ssDNA sequences derived from S. cerevisiae in water. The results obtained are encouraging, as 10 pM of DNA have been detected in only 5 minutes. Currently, we are using this technique to carry out tests on real samples. Specifically, we are detecting ssDNA release in the culture broth by S. cerevisiae during its growth.

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CO 12:

AN ECOFRIENDLY IRON MOF-BASED IMMUNOSENSOR FOR SENSITIVE DETECTION OF VASCULAR ENDOTHELIUM FROWTH FACTOR IN SERUM OF CANCER PATIENTS

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Metal-organic frameworks (MOFs) are a newly developing important class of multifunction crystalline porous materials that consist of connecting metal ions and organic linkers. MOFs exhibit high porosity, high surface areas, and tunable physical and chemical properties, demonstrating promise in a large-number of applications, such as gas storage, drug delivery, catalysis and sensing. However, their poor electro-conductivity may affect their extensive use in electrochemical (bio)sensing. Therefore, various strategies have been employed in order to overcome this limitation. In our work, we used as starting material MIL-100(Fe), a cheap, highly stable and bio-compatible MOF composed by trimesic acid and Fe3+ [1]. In order to tune the electroconductivity of MIL-100(Fe) electrochemical platform different synthesis reaction times (2-48h) have been tested. The morphological, structural and electrochemical properties of the different samples of MIL- 100(Fe) were evaluated using several physical and electrochemical techniques. The MIL-100(Fe) after 48 h owned a crystalline microporous-mesoporous structure with superior electrochemical properties, and therefore was used for the development of a novel immunosensor for sensitive detection of the vascular endothelial growth factor (VEGF) in human serum, with promising results [2]. Considering the sustainable and easy fabrication of the proposed platform, it may provide future application as a point-of-care (PoC) device for VEGF detection for early-stage diagnosis of cancer patients.

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CO 13:
CARCINOEMBRYONIC ANTIGEN DETECTION USING AN ELECTROCHEMICAL IMPEDANCE SPECTROSCOPY-BASED APTASENSOR

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Cancer is one of the most prevalent diseases globally and a leading cause of reduced life expectancy. Despite current diagnostic methods, there is a need for more precise and sensitive early detection techniques due to the increasing number of cancer cases worldwide. Aptamers, short nucleic acid molecules with high specificity and affinity for specific targets, offer an effective alternative to antibodies. They are simple to synthesize and modify, non-toxic, and stable over time. This study aimed to select single-stranded DNA aptamers against CEA (carcinoembryonic antigen) using the SELEX (systematic evolution of ligands by exponential enrichment) method. After 12 iterative SELEX rounds, nine aptamer candidates against CEA were developed. Comparative analysis using NGS showed that round 12 had an enriched number of specifically bound aptamers compared to round 8. One aptamer sequence with the highest specificity and affinity for CEA was identified and further examined in a concentration-dependent assay and specificity analysis. Additionally, its potential secondary and tertiary structures were predicted. Finally, this aptamer sequence was used in confocal microscopy to observe its binding towards CEA expressed in the HT-29 human colon adenocarcinoma cell line. An electrochemical impedance spectroscopy (EIS) based aptasensor was developed using the ssDNA aptamer identified in this study to detect CEA. The aptamer sequence was immobilized on an interdigitated gold electrode (IDE) surface modified with 18-HEG. The developed aptasensor demonstrated high specificity and sensitivity, with detection limits of 2.4 pg/ml and 3.8 pg/ml for CEA in buffer and human serum samples, respectively. The optimal incubation time for the target protein was 20 minutes, and EIS measurements took less than 3 minutes. Atomic force microscopy (AFM) micrographs confirmed the successful capture of the target, demonstrating a change in IDE surface roughness after each modification step. The potential of this developed EIS aptasensor in detecting CEA in complex samples holds promise.

CO 14:
BIOINSPIRED SYNERGY STRATEGY BASED ON THE INTEGRATION OF NANOZYME INTO A MOLECULARLY IMPRINTED POLYMER FOR IMPROVED ENZYME CATALYTIC MIMICRY AND SELECTIVE BIOSENSING

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Abstract

Nanozymes offer many advantages such as good stability and high catalytic activity, but their selectivity is lower than that of enzymes. This is because most enzymes have a protein component (apoenzyme) for substrate affinity to enhance selectivity and a non-protein element (coenzyme) for catalytic activity to improve sensitivity. To mimic this system, a combination of MIPs and nanozymes is proposed, where MIPs serve as the apoenzyme, providing substrate selectivity, and nanozymes act as the coenzyme, delivering catalytic functionality. This study integrates Fe_3O_4-Lys-Cu nanozymes, which exhibit peroxidase-like activity, into MIP specifically designed for L-DOPA. The MIP pre-concentration property boosted catalytic efficiency by a factor of twenty. Furthermore, the synergy between MIPs and nanozymes accelerated template removal during MIP synthesis, reducing the extraction time from several hours to just one minute. In parallel, the study addresses the issue of non-specific molecule binding, which is caused by functional groups outside the MIP cavities. To solve this, two surfactants: sodium dodecyl sulfate (SDS) and cetyl trimethyl ammonium bromide (CTAB) were used to prevent non-specific adsorption in MIPs. By modifying the MIP surface, the study improved selectivity, leading to more reliable and accurate sensing applications.

Keywords

Nanozymes, Molecularly Imprinted Polymers, Enzyme Mimicry, Selective Biosensing, supress of non-Specific Adsorption.

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CO 15:
INNOVATIVE MOLECULARLY IMPRINTED MEMBRANES WITH EFFECTIVE ELIMINATION OF NON-SPECIFIC ADSORPTION FOR ENHANCED SENSING APPLICATIONS

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Abstract

As the demand for rapid, green, and efficient synthesis of highly selective materials grows within sensing field, molecularly imprinted membranes (MIMs) showed a promising solution, by combining the selectivity of molecularly imprinted polymers (MIPs) with the versatility of membranes. In this study, bio-based MIMs were prepared using various synthesis techniques, including phase inversion and metal cross-linking. These MIMs were used as ready-to-use devices for smartphone-based on-site sensing of different templates. They showed exceptional performance in synthesis (simple, rapid, and green) and sensing (sensitive, reusable, performant filtration, and preconcentration) [1,2].

However, similar to MIPs, the MIMs encountered challenges with non-specific adsorption (NSA), which compromised their selectivity. To address this issue, an innovative modification, specifically designed for anionic-based MIMs was proposed. This approach, tested on sodium alginate and carboxymethyl cellulose-based MIMs, involved the crosslinking with calcium followed by phosphate chelating before template removal. In contrast to non-imprinted membrane (NIM), the developed MIM exhibited imprinting factor approaching infinity and a selectivity factor exceeding 100 times those reported in the literature. The resulting NSA-free MIMs exhibited excellent selectivity, sensitivity, and applicability in complex matrices. This innovative approach provides a potential source of inspiration for resolving NSA issues in a wide range of MIMs, applicable to various polymers and target molecules [3].

Keywords: Molecularly imprinted membrane; sodium alginate; cross-linking; non-specific adsorption; smartphone-based sensing.

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CO 16:

AN ELECTROCHEMICAL ENZYME BIOSENSOR FOR HYDROGEN PEROXIDE BASED ON POLY(SAFRANINE T)-TERNARY DEEP EUTECTIC SOLVENT AND CARBON NANOTUBE MODIFIED ELECTRODES

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Deep eutectic solvents (DES) are being explored as alternatives to aqueous media and ionic liquids in the formation of electroactive redox polymer films by electropolymerization on nanomaterial modified electrodes, with a view to improved performance in sensing applications [1,2]. DES are composed of at least one hydrogen bond acceptor (HBA) and one hydrogen bond donor (HBD) that, when mixed, form a new conducting eutectic phase characterized by having a lower melting point than its components (< 100 °C). Recent research has focussed on the exploring the potentialities of using ternary DES (two HBD) for electropolymerization instead of binary DES (one HBD) as in [3,4].
A new electrochemical enzyme biosensor has been developed to monitor hydrogen peroxide [5]. The biosensor uses a multiwalled carbon nanotube modified glassy carbon electrode covered by a poly(safranine T) film with immobilized catalase enzyme. Electropolymerization to form the polymer film was done by potential cycling in binary and ternary DES, prepared from choline chloride (ChCl) and ethylene glycol (EG) plus malonic acid (MA) or lactic acid (LA), with optimization of composition, and with H_2SO_4 as dopant acid. Electrochemical characterisation of the polymer films was done by cyclic voltammetry and electrochemical impedance spectroscopy, and the morphology was examined by scanning electron microscopy. The best electrochemical response was obtained with the ternary DES composition 16% ChCl:MA / 84% ChCl:EG. The enzyme biosensor was used for measurement of hydrogen peroxide in the concentration range from 1 to 4330 µM by fixed-potential amperometry, with a very low detection limit of 34 nM. Selectivity in relation to common interferents was found to be excellent and the biosensor showed a very good performance for hydrogen peroxide measurements in commercial products.

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CO 17:

A DNA-ELECTROCHEMICAL BIOSENSOR IN THE INVESTIGATION OF HAZARD COMPOUND-DNA INTERACTION MECHANISMS

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Many compounds bind and interact with DNA causing changes in the structure of DNA and in the base sequence, leading to perturbations in DNA replication. The need for the detection of oxidative damage and interactions of DNA with molecules led to the development of DNA-nanoscale electrochemical biosensors to investigate hazard compound-DNA interactions, elucidate the implications in the DNA structural modifications, and the mechanistic and cytotoxic aspects of their physiological action.

The DNA-nanoscale electrochemical biosensor consists of a device in which the DNA is immobilized on the electrochemical transducer surface to detect specific binding processes with different molecules, and to monitor electrochemical sensing of oxidative DNA damage.

It is also sensitive to changes in the DNA conformational structure after interaction with different hazard compounds, and the appearance of DNA base residues and their oxidation products, the biomarkers of DNA oxidative damage biomarkers 8-oxoguanine and 8-oxodeoxyguanosine [1-4]. The electrode is itself a tuneable charged reagent as well as a detector of all surface phenomena.

The interaction between different hazard compounds, pharmaceutical and chemotherapeutic drugs, and anticancer monoclonal antibodies, with a dsDNA-electrochemical biosensor, in incubated solutions using differential pulse voltammetry, was investigated. The drug-DNA interactions, and the occurrence of biomarkers 8-oxoguanine and 8-oxodeoxyguanosine, was also evaluated using dsDNA-, poly[G]- and poly[A]-electrochemical biosensors.

The changes in the dsDNA structural morphological conformation observed were related to the condensation and/or aggregation, distortion and/or unwinding of the double helix, binding of molecules in the DNA grooves, and release of guanine and adenine residues. Drug-dsDNA interaction mechanisms were proposed.

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CO 18:
DEVELOPMENT OF POTENTIOMETRIC SENSORS BASED ON SONOGEL-CARBON ELECTRODES FOR THE DETERMINATION OF NA+ AND K+ IN CLINICAL SAMPLES

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Clinical analyses provide indispensable and relevant information about the patient's conditions, but in many cases, it takes too much time to obtain the results. Therefore, it would be of particular interest to be able to carry out clinical analyses quickly and efficiently in primary care centres or even in the own patient's house. This would lead to a decentralization of clinical analysis, leading to a breakthrough in advance of digital healthcare (e-Health). For the decentralization of the system to take place miniaturization of the measuring is mandatory. In this vein, portable, selective and low-cost devices are becoming of great interest for the scientific and medical community. One of the most interesting point of view are the potentiometric sensors, in particular ion-selective electrodes capable of identifying species in a sensitive and selective way. In particular, K+ and Na+ ions, among others, are some of the parameters that are assessed in patients in intensive care units, with the aim of correctly monitoring their current health conditions.
In the present work, two potentiometric all-solid contact sensors for the determination of K+ and Na+ ions have been developed for biological samples application. The sensors are based on Sonogel-Carbon transducers, its surface modified by multi-walled carbon nanotubes that transduce the signal produced by the ions into electrons. Finally, a usual so-called membrane cocktail is added to provide adequate selectivity. This layer is made up of several species but the K+ ionophore I (valinomycin) and the Na+ ionophore X (4-tert-butylcalix[4]arene-tetraacetic acid tetraethyl ester), provide the selectivity.
The developed sensors provide good repeatability, reversibility and reproducibility results. The corresponding studies have also been carried out on the possible interferents present in blood serum samples, confirming the selectivity of both sensors. Analyses of serum samples were carried out in both batch and continuous regimes. A microfluidic cell previously developed in the research group was used to carry out the study in continuous regime. Finally, the K+ and Na+ concentrations determined by the respective and proposed sensor were validated by comparison with the reference value obtained by gasometry, which is the technique normally used in clinical laboratories.

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CO 19:
MULTIPOLYMER ELECTROCHEMICAL BIOSENSOR BASED ON SONOGEL-CARBON ELECTRODES FOR REAL-TIME LACTATE MONITORING: DECREASING COSTS IN TRANSDUCER AND FLOW CELL

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The study presents the development and evaluation of an innovative multipolymer-based electrochemical biosensor built on a Sonogel–Carbon electrode. The primary objective is to enable continuous and real-time determination of lactate in physiological samples. Lactate is a key biomarker in various clinical settings, including sports medicine, critical care, and metabolic disorders. Elevated lactate levels can indicate conditions such as hypoxia, sepsis, or mitochondrial dysfunction. Accurate and continuous monitoring of lactate is crucial for timely diagnosis and management of these conditions. This new approach leverages additive printing techniques to fabricate a versatile microfluidic cell, thereby enhancing the biosensor applicability in complex biological matrices.
The biosensor, created through a layer-by-layer modification process, demonstrates significant sensitivity and a broad linear range for lactate detection (0.2–20 mM). This is achieved by using a Sonogel–Carbon transducer, coated with lactate oxidase enzyme and multiple polymers. The device performance was validated through several tests:
Batch Mode Analysis: The biosensor exhibited excellent sensitivity (4.16 × 10-8 A·mM-1) and reproducibility, with a relative standard deviation (RSD) of 3.17% for repeatability and 6.36% for reproducibility. Stability tests over 8 hours showed no significant signal change, and the device maintained functionality for up to 15 days under storage conditions.
Continuous Mode Analysis: A microfluidic cell, designed via 3D printing, enabled the continuous monitoring of lactate. The calibration curve for lactate showed a high correlation coefficient ($R^2 = 0.9866$), though the sensitivity in continuous mode was lower due to reduced exposure time between the biosensor and the analyte. Tests with synthetic samples mimicking biological fluids confirmed the biosensor robustness, achieving recovery rates of 103 ± 2% for 2 mM and 95 ± 5% for 4 mM lactate concentrations.
The developed biosensor is a promising tool for continuous lactate monitoring in physiological samples. Its high sensitivity, stability, and ability to function in complex biological matrices underscore its potential for real-world applications. The innovative use of additive printing to create a customizable microfluidic cell enhances the biosensor adaptability and effectiveness in continuous monitoring scenarios.

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CO 20:

SIMPLE AND COST-EFFECTIVE pH AND T SENSORS FROM TOP TO BOTTOM: NEW CHEMICAL PROBES BASED ON SONOGEL-CARBON TRANSDUCERS FOR PLASMA ANALYSES

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The present work introduces two novel approaches to fabricate simple and cost-effective pH and temperature probes. Sinusoidal voltage methodologies were employed to electrodeposit polyaniline (PANI) at different growth times (10–20 min) on the surface of an affordable Sonogel-Carbon electrode to conform a robust pH sensor. The presence of PANI and its phases were corroborated by electrochemical means. The sensibility, reversibility and selectivity of the produced sensor were very adequate to apply it in physiological samples. In this regard, the proposed sensor was evaluated in artificial blood serum as well as untreated plasma samples obtaining outstanding results in comparison with a gold reference technique (error <2 %). In addition, a new composite sonogel material, intrinsically modified with multiwalled carbon nanotubes, was attached on top of an electrode couple to one-step fabricate a new temperature probe, relating resistance of the probe with the surroundings temperature. In this case, an optical microscopy characterization was performed to study the sturdiness of the layer. Remarkably, suitable results in terms of sensitivity and selectivity were obtained. The probes were assessed in artificial and untreated plasma samples as well, with the corresponding validation step (error <1 %) by using a commercial temperature probe.

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CO 21:

DEVELOPMENT OF ECO-FRIENDLY MOLECULARLY IMPRINTED POLYMERS USING DEEP EUTECTIC SOLVENTS FOR SELECTIVE EXTRACTION AND HPLC ANALYSIS OF PEPSIN: TOWARDS A BIOSENSOR FOR GERD BIOMARKER DETECTION

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This study presents the development of eco-friendly molecularly imprinted polymers (MIPs) using deep eutectic solvents (DES) as monomers and crosslinkers for selective extraction of pepsin enzyme from saliva, targeting its use as a biomarker for gastroesophageal reflux disease (GERD). Two DES systems were synthesised: one comprising methacrylic acid and cholin chloride as the functional monomer, and another with itaconic acid and [2-(Methacryloyloxy)ethyl]trimethylammonium chloride as the crosslinker. Polymerisation was carried out in aqueous buffer with no organic solvents. The MIPs exhibited a high binding capacity (Q, mg g-1) of 385 for pepsin, surpassing non-imprinted polymers (205 mg g-1). Optimisation strategies enhanced the binding efficiency and selectivity towards pepsin, with characterisation via FTIR, SEM, DSC, and TGA confirming their structural integrity and thermal stability. Selectivity studies demonstrated preferential binding to pepsin over competitor enzymes like amylase and lipase. The developed MIPs were applied successfully for pepsin extraction from human saliva samples, followed by quantification using a size-exclusion chromatography method integrated with high-performance liquid chromatography (HPLC-SEC). This integrated system of MIPs with HPLC analysis serves as a biosensor capable of quantifying pepsin within a linear range of 1 to 150 µg mL-1, showcasing its potential as a sensitive tool for GERD biomarker analysis and highlighting DES-based MIPs as promising materials in bioanalytical chemistry and biomedical research.

CO 22:
DEVELOPMENT OF A CONTINUOUS LEVODOPA MONITOR FOR IN VIVO APPLICATION

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Our group focuses on integrating novel molecular recognition elements, with new electrochemical detection modalities, for the detection and monitoring of various substrates. One pillar of this work is on the development of direct electron transfer (DET) type enzymes, which can be integrated into sensors using electrochemical techniques, such as open circuit potential, chronoamperometry, or voltammetry. Using these techniques, we have reported several enzymatic biosensors for continuous monitoring which will be utilized for the enhancement of 2,3 the therapy across a variety of disease states.
One example of this is Parkinson's Disease (PD), a neurodegenerative disease caused by 4 the breakdown of dopaminergic neurons within the substantia nigra . This leads to insufficient production of dopamine, causing debilitating symptoms, including tremors, stiffness, and bradykinesia. Levodopa remains the first line therapy, supplanting the lack of endogenous dopamine. While effective, levodopa therapy is far from ideal, requiring a tight therapeutic window, with poor outcomes caused from either over/under dosing. To better treat patients, the idea of a levodopa sensor has gained interest, which could help patients and clinicians better 5 titrate levodopa, and aid in the development of precision therapies .
Recently we have developed a continuous levodopa monitoring (CLM) system based on an engineered DET enzyme. By integrating this DET enzyme with a unique, pulsatile electrochemical protocol (open circuit potential and chronoamperometry), we have achieved strong sensitivity and specificity over the levodopa therapeutic window. This CLM was characterized in vitro, for specificity against over 25 endogenous/exogenous targets and sensitivity across the therapeutic window (0 – 55 μM), followed by validation using ex vivo human plasma samples. The sensor gave a limit of detection of 178 nM in human plasma, with a dynamic window from 0.3 – 55 μM, encapsulating the entire therapeutic range. Finally, the CLM has been tested in vivo using Sprague Dawley rats, demonstrating for the first time, a CLM system which may be used in vivo.

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CO 23:

DETECTION OF SINGLE NUCLEOTIDE POLYMORPHISMS IN HUMAN GENOMIC DNA USING ELECTROCHEMICAL BIOSENSOR BASED ON MELTING ANALYSIS

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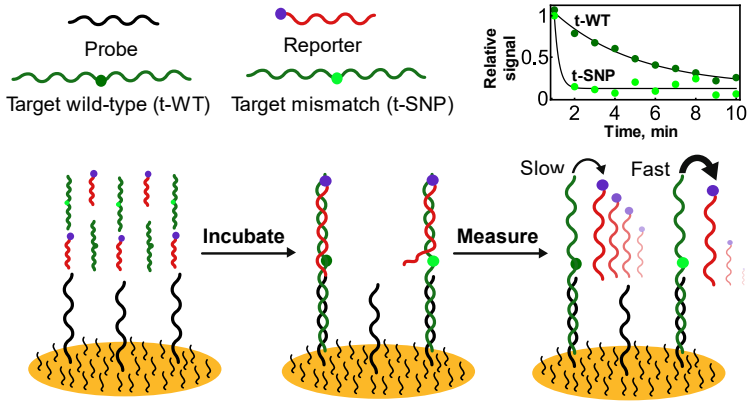

Single nucleotide polymorphisms (SNPs) are the substitution of a single base pair at a specific location in a genome. SNPs are the most common variation in the human genome, occurring on average once every 300 base pairs with a minor allele frequency greater than 1%, [1] and over 84 million SNPs have been identified. [2] While most SNPs are harmless, some can lead to severe pathological changes, e.g., aging processes, [3] and their detection is of great importance. Current traditional methods for SNPs detection include TaqMan SNP genotyping and SNP microarrays, indicating the need to develop rapid and more robust methods for SNPs detection. Here, we report a single-electrode biosensor for rapid and simple detection of SNPs in human genomic DNA directly after PCR amplification. As a proof-of-principle, a biosensor was designed to investigate SNP of a pharmacogene belonging to the cytochrome P450 family – CYP2C19. The biosensor was designed to detect the CYP2C19*17 (SNP17) allele, which is responsible for the "gain of function" that significantly alters the pharmacokinetics of important drugs (e.g., clopidogrel). [4] Our designed biosensor was fabricated using a "sandwich" principle: a gold electrode was coated with probe DNA, followed by hybridization to a target (wild-type or SNP17, or their mixture) and subsequent hybridization of methylene blue (MB)-labeled reporter DNA, which is fully complementary to the wild-type target. The biosensor exploited the differences between the melting kinetics of wild-type and SNP17 nucleotides by measuring the dissociation of the MB-labeled probe from the surface. We demonstrated significant melting differences between wild-type and SNP alleles (relative kD=0.1 s–1 and 21.2 s–1 for wild-type and SNP17, respectively), allowing the biosensor to discriminate between homozygous (wild-type or SNP17) and heterozygous (wild-type and SNP17) samples.

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CO 24:

REAL-TIME C-REACTIVE PROTEIN MONITORING VIA FIBER OPTIC SENSOR AND MACHINE LEARNING IN WASTEWATER

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The research presents an optical method for real-time wastewater analysis. C-Reactive Protein (CRP) is inflammation biomarker, sensitive to any form of inflammation. The monitoring changes carries important information for early detection during epidemic in communities of concern, such as hospitals, schools and offices.
Presented fiber optic based sensor monitors in real-time the dynamics of CRP changes in wastewater. The sensor were made with infrared source, spectrum analyzer and a standard telecommunications optical fibre with modified sensor head into a microsphere covered with additional biofunctionalized layer. Used machine learning supports the detection of extraordinary increased amounts of CRP and allows early warming of epidemic. Measurements were validated on samples with different CRP concentrations in both urine and wastewater. The sensor is characterized by simplicity, mobility and versatility, which make it suitable for implementation in existing wastewater infrastructure. This would allow monitoring of large areas.

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CO 25:
URINARY MASS SCREENING WITH OPTICAL METHOD SUPPORTED BY MACHINE LEARNING

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The research aims to presents an optical method for rapid urinary sample differentiation, specifically designed for mass screening. The method comprises spectra of absorption spectroscopy measurement of patients' urine samples with: urosepsis, urinary tract infection and bladder cancer. Based on the obtained spectroscopy measurements the classification is performed using machine learning to target infections capable of causing urosepsis or urinary changes suggestive of bladder cancer.

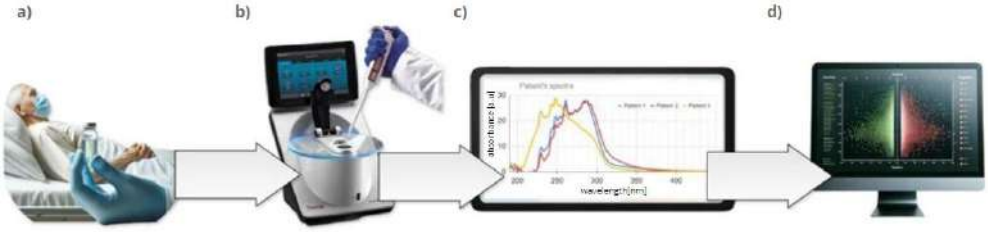

Figure 1: Steps of the proposed method: a) Collecting samples, b) Conducting optical measurements, c) Analysis of absorption spectra, d) Classification with ML

The method was validated on urine samples from 150 unhealthy patients. We proved that is possible to obtain up to 94% accuracy of the measurement method with the support of machine learning. The optical method can support current method of detection of a life-threatening condition such as urosepsis. The advantages of the proposed solution are the simplicity of the sensor, mobility, versatility, and low cost of the test, which allow to implement the method on a large scale.

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CO 26:
POLYMER FILM MODIFIED ELECTRODES PREPARED IN DEEP EUTECTIC SOLVENTS FOR SENSORS AND BIOSENSORS

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Deep eutectic solvents (DES), alternatives to room temperature ionic liquids and to non-aqueous solvents and water, are media for electrodeposition, nanomaterial formation and, more recently, the preparation of sensors. Formation of the eutectic, by simple direct mixing of the components, usually both solid and in the correct proportions, occurs due to strong hydrogen bond interactions between a hydrogen bond acceptor (HBA), such as choline chloride, and a hydrogen bond donor (HBD) such as urea or ethylene glycol. They have found wide applications in many areas of materials science, and more recently in sensors [1].

These binary DES have been used by us for the preparation of polymer films on nanomaterial-modified electrodes, for application in sensors and biosensors. The polymer film properties can be enhanced by the addition of small amounts of strong acid dopants to the DES. Another enhancement strategy to explore is to develop ternary DES that contain two HBD which opens up a wide range of possibilities.

The latest research in the development of these modified electrodes for sensing and biosensing prepared in binary and ternary DES will be highlighted. The polymers have been phenazine or triarylmethane redox conducting polymer films, together with nanoparticles of gold or carbon nanotubes on glassy carbon electrode substrates. These will be described with emphasis on poly(methylene blue) [2], poly(neutral red) [3], poly(crystal violet) [4], and poly(methylene green) [5] in ternary DES. Excellent results have been obtained for environmental and pharmaceutical analytes with high selectivity and low detection limits. Future perspectives will be indicated.

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CO 27:
CONTROLLABLE LONG-DISTANCE DROPLET TRANSPORT WITH A BIOMIMETIC 2D EWOD ELECTRODE

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Recently, numerous studies have reported that insect legs possess unique physical structures enabling efficient droplet transport. Consequently, many biomimetic 3D structures have been proposed for long-distance directional droplet transport in microfluidic systems. However, these 3D structures often face challenges such as integration difficulties, sample loss, and transfer issues, etc. In contrast, Electrowetting on Dielectric (EWOD) manipulates droplets by altering the wettability of the chip surface via changes of electrode potential, providing a new solution to the problems.
This study flattens the morphology of insect legs and setae, proposing a 2D biomimetic EWOD electrode for long-distance directional droplet transport, see Figure 1. When a droplet is on the electrode, it experiences continuous unbalanced wettability forces as the driving force. The study found that the contact line length of the droplet on the electrode, the magnitude of the driving force, and the average speed of droplet movement are all linearly correlated with the key parameter α of the electrode. This implies that the droplet's movement on the electrode is predictable and controllable. A model is proposed to predict the droplet movement on the biomimetic electrode. Thanks to the design of such electrode, long-distance transport can be achieved without complex control systems, numerous physical electrodes, and/or extensive wiring, greatly enhancing the integration possibility of microfluidic systems.

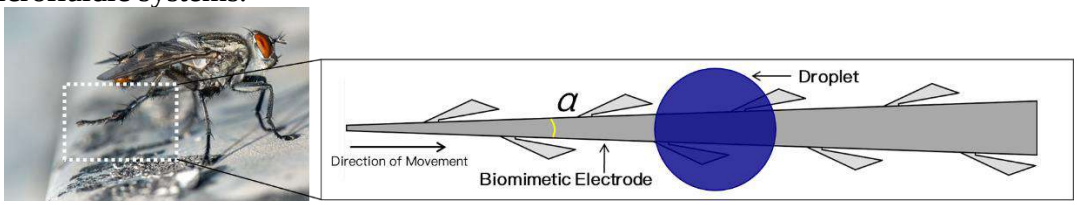

Figure 1. Structure diagram of 2D biomimetic electrode

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CO 28:
BIOELECTROCHEMICAL DEVICES BASED ON BUCKYPAPERS

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For four decades, the functionalization of electrodes by biomaterials based on electrogenerated polymers, carbon nanotubes and / or nano-objects, was widely used in the field of analytical chemistry and energy conversion for the design of biosensors and biofuel cells [1].
The self-assembly of carbon nanotubes in the form of conductive sheets (buckypapers) was used to generate bioelectrodes. We, thus, reported the modification of carbon nanotubes with both alkyne-modified diazonium salts and alkyne-modified pyrene followed by photo-irradiated thiol-yne click reaction with thiol-modified redox mediators. The resulting carbon nanotubes achieve the mediated bioelectrocatalytic glucose oxidation by adsorbed FAD-glucose dehydrogenase [2].
Recently, the concept of hollow bioelectrodes based on the bonding of two buckypapers was developed to generate a microcavity defined by the thickness of the glue linking the two sheets. These buckypapers are permeable only to water and enzyme substrates but not allow the permeation of enzymes. Therefore, the enzyme trapped in powder form is then solubilized inside the microcavity leading to a high density of biocatalyst in solution with an electrical connection with the buckypapers. The electrocatalytic performance of the bilirubin oxidase hollow electrode was described as a function of pH, temperature and the amount of entrapped enzyme [3].
Owing to the complexity of optimizing a multienzyme system, this concept also constitutes an attractive strategy to design and optimize enzyme cascade reactions. Besides the easy modulation of enzyme ratios, we have also demonstrated the possibility of trapping with enzymes a redox mediator ensuring the electrical connection of an enzyme. Following this strategy, the immobilization of HRP and GOx in a buckypaper microcavity, along with a redox mediator (ABTS) led to an efficient bioelectrode for the glucose sensing at 50 mV vs Ag/AgCl. The bienzyme electrode demonstrates continuous operation for over a week and excellent storage stability, retaining 71% of its initial sensitivity even after 100 days [4].
It is expected that such construction and fast optimization of multienzyme electrochemical systems based on hollow electrodes, will be useful for the development of biosensors and biofuel cells.
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CO 29:
NANOSTRUCTURIZED ELECTRODES FOR ULTRASENSITIVE DETECTION OF BIOMARKERS

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Early diagnosis of disease is crucial for successful treatment. By identifying the onset of a disease, it is possible to prevent its spread to other parts of the body or to other people. Rapid disease diagnosis helps the patient retrieve before the critical condition and reduces the doctor's burden for prescriptions. The main challenge in early diagnosis is that symptoms are often mild, and biochemical changes in the body are barely detectable at the initial stage. Therefore, ultrasensitive and point-of-care analytical methods are essential for successful treatment at the early stages of diseases. Electrochemical sensors are particularly suitable for this purpose since they enable rapid detection while maintaining high sensitivity, selectivity, and low limit of detection. The combination of these features, along with the capability for repeated measurements, allows for the monitoring of subtle changes in biomarker concentrations over time, thus reducing diagnosis time and increasing treatment efficiency [1,2].
Recent studies highlight the usefulness of electrochemical sensor research for ultrasensitive diagnosis, especially when utilizing advanced nanomaterials. The aim of this presentation is to share the recent achievements of the Bioelectrochemistry Group in developing nanostructured electrodes-principles detecting specific biomarkers with high selectivity and sensitivity. These developments include microporous conducting polymers, functionalized MWCNTs, carbon-based nanomaterials, gold nanostructures, MOF, and MXene-based materials, that were functionalized to serve as sensitive detection tools for Carcinoma antigen 125, C-reactive protein, Procalcitonin, Tumor necrosis factor-alpha, High-mobility group box 1 protein, and Interleukin-6, associated with ovarian cancer and sepsis. These materials exhibit unique physicochemical properties and electroactivity, enabling their successful implementation in biomedical research.

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CO 30:
A NANOPAPER-BASED SMART DUAL-BIOSENSOR FOR REAL-TIME OPTO-ELECTROCHEMICAL ON-FIELD DETECTION OF ORGANOPHOSPHORUS PESTICIDES

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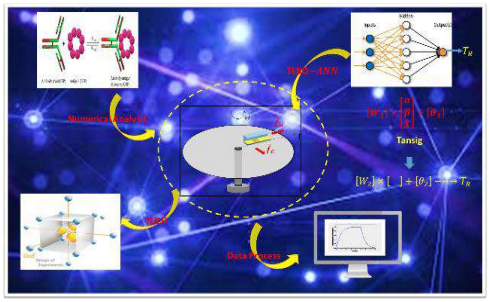
The use of pesticides is a common practice in the agriculture industry to increase the yield of crops. Among the others, the excessive exploitation of organophosphorus pesticides (OPs) is an ever-growing concern regarding their implications for environmental protection and public health. To promptly address the adverse impacts of pesticide use, developing user-friendly, affordable, and reliable, analytical platforms for in-situ monitoring of OPs is of primary concern.
Herein, using nano-cellulose paper as a novel sustainable nanoplatform, we developed a reagent-free, opto-electrochemical smart device for on-site detection of paraoxon, an OP model.
In detail, the nano-paper-based analytical device (PAD) consist of i) a bacterial cellulose nanopaper where the electrochemical cell was screen printed, and ii) a semi-transparent TEMPO-oxidized cellulose nanopaper, where the optical detection takes place. Furthermore, the nanopapers were wax printed to delimitate the areas for the enzyme and enzymatic substrates pre-loading, delivering a reagent-free device.
The detection principle relies on the paraoxon inhibition of the enzymatic reaction of butyrylcholinesterase with i) butyrylthiocholine, suppressing the thiocholine electroactive product detected by amperometric technique, and ii) indoxyl acetate, decreasing the indoxyl fluorescent product measured by color intensity.
Additionally, the device was integrated with a smartphone-based miniaturized potentiostat alongside a self-developed portable optical reader and Android application.
After optimization of various parameters, including substrate concentration, enzyme concentration, and reaction time, the device was tested at different paraoxon concentrations (linear range equal to 20-100 ppb). Calibration curve equations equal to $Y = (2.0 \pm 0.2)\ X - (27 \pm 8)$, R^2 of 0.973, and $Y = (0.72 \pm 0.04)\ X + (4.0 \pm 2.5)$, R^2 of 0.995, were obtained for the electrochemical and optical detection, respectively.
Finally, the accuracy of the device was assessed by analysis of untreated wastewater samples, obtaining acceptable recovery percentages in the range of 90-110%, demonstrating our system's applicability for environmental surveillance.



CO 31:
OPTIMIZING LAB-ON-A-CD BIOSENSOR FOR CANCER DIAGNOSIS BASED ON CRP MARKER DETECTION

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C-reactive protein (CRP) emerges as a promising pre-diagnostic indicator for lung cancer, particularly when accompanied by additional symptoms, providing a valuable cue for examining individuals at high risk. This study employs a rotating microfluidic biosensor for the CRP marker to investigate the centrifugal and Coriolis effects arising from the angular alignment and radial displacement of the biosensor. Applying the finite element method (FEM), a two-dimensional system of equations is solved, encompassing the Navier-Stokes, analyte transport, and binding reaction equations. The impact of input variables, such as rotational velocity (20-100 rad/s), biosensor position (150-350 µm), angular alignment (0-90°), and radial displacement (2-10 mm), was systematically explored using Behnken design (BBD) and the teaching-learning-based optimization of an artificial neural network (TLBO-ANN). The global desirability function was employed to determine the optimal detection time, found to be 1.23 minutes, achieved when the rotational velocity was 83 rad/s, biosensor position was 350 µm, angular alignment was 31.8°, and radial displacement was 10 mm. Notably, this result was accurately predicted by BBD (0.83 min) at a confidence level of 95%, affirming the precision of our model predictions. Additionally, the R2 and MSE values for BBD were determined to be 93.27% and 4.3572, respectively, while those for TLBO-ANN were 99.9542% and 0.0013. Based on the higher R2 value and lower MSE, it can be concluded that TLBO-ANN outperformed BBD in terms of predictive accuracy. This comprehensive approach not only advances our understanding of CRP as a diagnostic tool but also demonstrates the efficacy of our study in the optimization of the biosensor detection parameters for enhanced precision in simple, cost effective and real time, early-stage lung cancer diagnosis.
Keywords: Lab-on-a-CD, Biosensor, CRP, TLBO-ANN, Lung Cancer Diagnosis.
(SMARTANTICANCER).

CO 32:

A MACHINE LEARNING APPROACH FOR SUSTAINABLE, SMART AND PORTABLE SEROTONIN AND DOPAMINE SIMULTANEOUS ANALYSIS NANOPLATFORM DEVELOPMENT

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Dopamine (DA) and Serotonin (5-HT) are two key neurotransmitters with profound impacts on health and behavior. The dysregulation of their levels is associated with a several diseases, which makes the simultaneous quantification of both essential for the understanding and treatment of such conditions. We have succeeded, in the development of Screen-printed electrodes (SPE) based Electrochemical (EC) simple, cost-effective and portable sensors for the simultaneous detection of dopamine (DA) and serotonin (5-HT), using silver nanoparticles (AgNPs) decorated reduced graphene oxide (rGO). The sensor's performance was evaluated using differential pulse voltammetry (DPV) and cyclic voltammetry (CV) techniques and were optimal at pH 7.1, demonstrating high selectivity and sensitivity, with detection limits of 7 and 7.4 μM for DA and 5-HT respectively.
In this work, we incorporate machine learning (ML) methods, including linear regression, decision trees, random forests, AdaBoost, and gradient boosting, to enhance prediction accuracy for the concentrations of DA and 5-HT separately. This approach aims to further improve the sensor's capabilities by distinguishing both of neurotransmitters in a single measurement, thereby enhancing its application potential in clinical diagnostics. Thus, the results show, that the tree-based models, particularly random forests and gradient boosting, significantly outperform other methods in terms of prediction accuracy and error rates. The random forest model achieved error rates of 3.5%, and 4.2% for dopamine and serotonin detection respectively. Principal Component Analysis (PCA) was implemented to reduce data dimensionality, enhancing the accuracy of the predictions. The database used for training and testing the models comprised comprehensive electrochemical response data, ensuring robust model performance.
In summary, the integration of ecofriendly nanomaterials and Green AI techniques in the sensor design provided a robust nanoplatform for the detection of multiple bio analytics demonstrating the feasibility and effectiveness of this innovative methodology, paving the way for future advancements in electrochemical sensing technologies and their biomedical applications.

Keywords: Machine learning, Sustainable, serotonin, dopamine, simultaneous analysis

CO 33:
FUNCTIONALIZED ORTHOPAEDIC IMPLANT AS pH ELECTROCHEMICAL SENSING TOOL FOR SMART DIAGNOSIS OF HARDWARE INFECTION

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Most of current surgical treatment in orthopaedic field aim to fix a patient's bone issue, such as fractures, implanting a medical hardware. However, this widespread practice provides high odds of infection which can lead to device failure and long-term hospitalization, mostly based on expensive antibiotic therapies. The infection onset is closely linked to the bacterial adhesion on hardware surface, resulting in a pH decrease from physiological level to more acidic pH values.
In this regard, here we developed an innovative strategy focused on using an iridium oxide-functionalized orthopaedic implant as a tool for potentiometric monitoring of pH in hardware infection diagnosis.
After selecting the implant material among the ones which have been functionally assessed, such as titanium, titanium alloy and stainless steel, as well as the component like screws and implants, we set up the final sensing configuration, characterized by titanium-based implant as working electrode and a silver wire as reference electrode. Then, a calibration curve was performed in standard solutions, obtaining an equation equal to $y = (0.76 \pm 0.02) - (0.068 \pm 0.002)\ x$, $R2 = 0.996$, in the pH range 4-8.
Furthermore, the sensing device's overall performance was evaluated going through several analyses as following: hysteresis, interference, matrix effect, recovery study and storage stability. These investigations pointed out the huge capability of electrochemical sensors to move next generation of smart orthopaedic implants forward.

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CO 34:
RAMAN SPECTROSCOPY IN ANALYSIS OF PIGMENTS IN SKIN: APPLICATIONS IN TATTOO SAFETY

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With the growing popularity of tattoos, understanding their safety and composition is crucial to preventing and addressing complications.
We used Raman spectroscopy to analyze tattoo pigments in tissue and tissue phantoms. Our goals were: (1) non-invasive measurement of pigment in the skin and (2) the use of Raman spectroscopy with skin phantoms to determine the potential risk of using a given pigment.
We created and measured using Raman spectroscopy specialized phantoms mimicking tattooed skin. Unlike past research on tattoo inks, this study focused on pigments remaining in healed tattoos. Accurately replicating the chemical composition of a healed tattoo was essential for realistic skin models. We also tested phantoms for imitating the tattoo removal by processing them with a dermatological laser.
The results indicate that Raman spectroscopy is a promising method for analyzing tattoo pigments in tissue, also with potential applications in risk assessment of using pigment, before introducing it to the market.

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CO 35:
PAPER-BASED ELECTROCHEMICAL POINT-OF-CARE DEVICE FOR PRECISION MEDICINE IN BREAST CANCER

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Real-time updates regarding the health condition of individuals have become a prominent subject in contemporary medical research activities. Undoubtedly, the ultimate objective is to shift from traditional hospitals to a home-based medical care approach to enhance the overall healthcare system1. Technological advancements are playing a pivotal role in facilitating this significant transition, as they allow for the development of compact sensing devices, which can replace cumbersome instruments, eliminate uncomfortable wire connections, and deliver straightforward data-transferring procedures based on wireless transmission2. In this regard, paper-based technology is an obvious choice as a support material, thanks to its wide range of outstanding properties, such as low cost, eco-sustainability, versatility, affordability, and biocompatibility. Indeed, paper can be used to fabricate microfluidic systems integrated into point-of-care devices, conferring several fascinating features to these smart analytical tools. Herein, we present an innovative point-of-care platform for the home-based self-assessment of haemoglobin and electrolytes in capillary blood to support oncology patients at home. The point-of-care platform was combined with a four-leaf clover paper-based microfluidic system to manage the solution, creating a hydrophobic zone to define a hydrophilic working area. The sensing tool encompasses a double-printed electrochemical sensor for haemoglobin detection and three ad-hoc modified printed sensors connected with a multi-chip NFC system consisting of multiple boards for wireless simultaneous detection of the target analytes. The electrochemical performance of the haemoglobin sensor has been tested directly in the real matrix, analyzing more than 40 capillary blood samples and constructing a calibration curve described by the following equations: $y = 0.1x + 0.7$, $R2 = 0.884$. Moreover, the ionophore-based carbon black-potentiometric sensors for Na+, K+, and Ca2+ have been tested in standard solution obtaining calibration curves described by the following equations: $y = (0.056 \pm 0.002)\ x + (0.300 \pm 0.004)$, $R2 = 0.993$, $y = (0.057 \pm 0.001)\ x + (0.595 \pm 0.003)$, $R2 = 0.986$, and $y = (0.034 \pm 0.001)\ x + (0.319 \pm 0.001)$, $R2 = 0.997$, respectively. The applicability of this sensing platform was evaluated by measuring capillary blood samples of breast cancer patients, obtaining results in agreement with the reference method.

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CO 36:
SENSITIVE AND SELECTIVE DETECTION OF TOBRAMYCIN IN FOOD SAMPLES USING SILVER NANOPARTICLES-ENHANCED MOLECULARLY IMPRINTED POLYMER SENSOR AND ELECTRONIC TONGUE

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Tobramycin (TOB) is an aminoglycoside antibiotic for treating chronic lung infections and other diseases. TOB residues can be found in feed from treated animals, leading to antibiotic resistance in consumers [1-3]. This study developed tools for TOB detection using a multi-sensor (electronic tongue) and an electrochemical sensor based on a conductive polymer with molecularly imprinted polymer (MIP). The MIP sensor, made of polyaniline on a screen-printed gold electrode (Au-SPE) and enhanced by silver nanoparticles, was verified using SEM and FTIR. Cyclic voltammetry (CV), differential voltammetry (DPV), and electrochemical impedance spectroscopy (EIS) were employed during the sensor preparation and TOB detection phases. The sensor achieved a detection limit of 1.9 pg/mL over a range of 0.001 pg/mL to 60 pg/mL. The selectivity of the MIP sensor was proven in samples of chicken meat, beef meat, turkey meat, eggs, and milk. As TOB was highly present in the milk samples, these were analyzed using the electronic tongue and chemometric methods. Using Principal Component Analysis (PCA) and Discriminant Function Analysis (DFA), the electronic tongue could distinguish between milk samples without TOB and those with varying concentrations. PCA showed 96.94% of the total variance of the data.
Keywords: Tobramycin; Electrochemical sensors; Molecularly imprinted polymer; Electronic Tongue; Food.

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CO 37:
CONTROLLED SURFACE BIOFUNCTIONALIZATION OF GRAPHENE FIELD-EFFECT TRANSISTOR BIOSENSORS AS A POWERFUL TOOL TO IMPROVE SENSITIVITY AND REPRODUCIBILITY IN LABEL-FREE SARS-COV-2 DETECTION

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The COVID-19 global crisis has underscored the critical necessity for a swift, highly sensitive diagnostic tool. The integration of two-dimensional nanomaterials, especially graphene, into portable medical devices stands as a pioneering approach poised to transform diagnostics profoundly. Despite progress in crafting these biosensors, the link between their surface modification and sensing efficiency remains uncertain. Here, we illustrate how meticulous sensor production and precise surface modification is crucial in augmenting the capabilities of two-dimensional biosensors. Specifically, we have biofunctionalized Graphene Field-Effect Transistor (GFET) sensors surface through different biochemical reactions to promote either random/heterogeneous or oriented/homogeneous immobilization of the Anti-SARS-CoV-2 spike protein antibody. Each approach underwent comprehensive analysis via computer simulations, physical and biochemical methods, and electrical assessment. Following this, both biosensors were evaluated for their capacity to directly detect SARS-CoV-2 virus in simulated clinical samples without sample preparation and accomplishing rapid results. Notably, the oriented GFET biosensor exhibited significantly enhanced reproducibility and responsiveness, surpassing the detection sensitivity of conventional non-oriented GFET by over twofold. This advancement not only holds immediate implications for COVID-19 monitoring and future pandemic readiness but also sheds light on an unexplored aspect of biosensor research utilizing two-dimensional nanomaterials.

Keywords: Graphene Field effect transistor, biosensor, oriented immobilization, two-dimensional materials, nanobiotechnology, antibody

Reference: Lozano-Chamizo L, Márquez C, Marciello M, Galdon JC, de la Fuente-Zapico E, Martinez-Mazón P, Gonzalez-Rumayor V, Filice M, Gamiz F. High enhancement of sensitivity and reproducibility in label-free SARS-CoV-2 detection with graphene field-effect transistor sensors through precise surface biofunctionalization control. Biosens Bioelectron. 2024, 250,116040.

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Poster Communications

PO 01:

SOFT LASER ABLATION AND FTIR SPECTROSCOPY: TOWARDS RAPID IDENTIFICATION OF PATHOGENIC BACTERIA IN THE AGRI-FOOD INDUSTRIES

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Current approaches to bacterial identification are generally based on periodic sampling followed by culture on selective media and/or the use of an identification technique. Polymerase chain reaction (PCR), for example, is a technique based on the amplification of a specific gene of a target pathogen. However, despite its rapidity, PCR requires rigorous laboratory conditions to avoid any inhibition of amplification by the water used in the agri-food industries. Advances in laser technology have provided rapid bacterial identification tools, such as those employed in matrix-assisted laser desorption-ionization time of flight (MALDI-TOF)[1] mass spectrometry, recently introduced to hospitals in Europe, Canada, and USA. This has enabled rapid diagnosis and identification of many infectious bacteria. The MALDI used at the "Centre Hospitalier Universitaire de Sherbrooke" can identify up to 192 bacteria at a time in around 2 hours. However, this technique requires a relatively high bacterial concentration, moreover, the high cost of instrument acquisition limits the application of MALDI technology to laboratories in developed countries. In this project, we aim to develop an innovative method based on soft laser ablation and Fourier transform infrared spectroscopy (FTIR) for the rapid and sensitive identification of pathogenic bacteria in the agri-food industries. To achieve this, we propose to apply a UV laser ablation on matrix-treated bacterium to transfer the biological materials to an IR-transparent target (Figure 1). The ultimate goals are (i) to develop a fast and low-cost identification tool, enabling generating a specific spectral fingerprint assigned to each targeted bacterium, and (ii) to reduce detection/identification time by analyzing directly collected samples and concentrated using the Water Sampling Module (WSM)[2] developed at the Université de Sherbrooke.

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[2]K. Moumanis et al., Water Sampling Module for Collecting and Concentrating Legionella pneumophila from Low-to-Medium Contaminated Environment; Biosensors; 11(2), 34 (2021).

PO 02:

EXPLORING THE ROLE OF MODULABLE BIOSENSING SOLUTIONS IN ONE HEALTH EFFORTS FOR MANAGING GLOBAL HEALTH CHALLENGES IN MEDICAL DIAGNOSTICS AND ENVIRONMENTAL MONITORING

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In light of ongoing global health challenges, this poster presents practical modulable biosensing solutions that facilitate the rapid and cost-effective detection of nucleic acids for applications in medical diagnostics and environmental monitoring. First is an electrochemical DNA biosensor that utilizes a phosphoramidate-bonding strategy for immobilizing non-modified single-stranded DNA on a low-cost pencil graphite electrode modified with gold nanoparticles and carbon black. This approach is particularly cost-effective, being up to 10 times less expensive than conventional methods as it allows for the direct immobilization of capture probe sequences without the need for additional chemical modifications such as thiol (-SH) groups. Through nothing more than an easily accessible label-free detection method this biosensor provides good results with a linear range from 10 nM to 500 nM and a detection limit of 1 nM for microRNA-21. Following this is a dual-mode biosensor that incorporates Exonuclease III-assisted target recycling amplification with a high-throughput 96-well microplate format. This ready-to-use bioplatform offers a cost-effective pathway by employing a phosphoramidate-bonding strategy as an alternative to the common immobilization approach used in 96-well microplate format. The integration of target recycling amplification enables this ready-to-use bioplatform to perform well in colorimetric and electrochemical detection achieving good results with a linear range from 100 fM to 100 nM and a detection limit of 1 fM for Hepatitis B virus DNA. In this last approach a biosensor employing laser-scribed graphene electrodes is designed for the detection of Escherichia coli using a sandwich hybridization system. The electrodes are created using a simple diode laser enabling affordable and accessible production. Magnetic beads with specific nucleic acid probes capture and concentrate target bacteria improving overall electrochemical detection capabilities. This comprehensive set of approaches provides cost-effective methods for medical diagnostics and environmental monitoring through modulable biosensors to address health challenges.

Keywords: One Health ; Modulable Biosensing ; Nucleic Acid Testing ; Cost-Efficient Methods.

PO 03:

NOVEL STRATEGY FOR LEAD DETECTION IN WATER SAMPLES USING Si_3N_4 WAFER COATED WITH IONOPHORE-DOPED CHITOSAN NANOFIBERS.

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Lead is the most abundant heavy metal in the Earth's crust. Its unique properties [1], such as ductility, softness, and resistance to corrosion, have made it a widely used metal in various industries. However, lead is also a harmful environmental pollutant which can induce neurological, respiratory, urinary and cardiovascular disorders. According to the World Health Organization [2] in 2019, half of the two millions death caused by chemical exposure were due to lead exposition and contamination from pipes, lead paint and residual emissions from leaded gasoline. Currently, the main ways of detection of lead are performed by inductively coupled plasma mass spectrometry (ICP-MS), atomic absorption spectrometry (AAS) and portable X-ray fluorescence (XRF), which are expensive methods requiring trained people to conduct measurements. The aim of this work is to develop a novel ultra-sensitive, cost-effective, and easy-to-use sensor based on a Si_3N_4-substrate for in-river monitoring. The sensor is produced by capturing tert-Butylcalix[4]arene-tetrakis(N,N-dimethylthioacetamide), referred as lead ionophore IV, between the substrate and a layer of electrospun chitosan nanofibers—a biodegradable and biosourced polymer. The Si_3N_4 surface was activated via functionalization with 11-(Triethoxysilyl)undecanal (TESUD). After optimization, a chitosan solution doped with the tert-Butylcalix[4]arene-tetrakis(N,N-dimethylthioacetamide) was electrospun onto the aldehyde-terminated Si_3N_4 surface. SEM imaging revealed a promising fiber structure: bubble-free and with homogeneous fiber diameter. This structure can be used for lead detection as well as for detecting any molecule that has an affinity with chitosan. The sensor was tested for the detection of lead in artificial acid water solutions without any interferent and demonstrated responsiveness to lead and resistance to mild acidity.

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PO 04:
PRINTABLE MEDIATED GLUCOSE BIOSENSOR DEVELOPMENT FOR WEARABLE DEVICES

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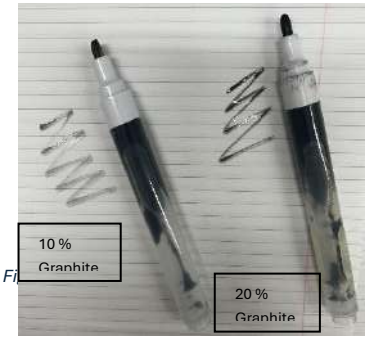
A multiparametric, non-invasive and reagentless sensing strategy for diabetic monitoring is proposed based on a bespoke graphite ink "writable" formulation (including biocompatible binders and modifiers, Gink) (Fig. 1) as conductive layer for biocatalyst immobilisation within an epidermal patch. This enables encapsulation of the heterocyclic quinoid species 1,10-phenanthroline-5,6-dione which acts as a proton and electron acceptor for FADH2 cofactor regeneration of glucose oxidase. Optimisation and characterisation of the Gink on glassy carbon electrodes involved electrochemical, surface and optical investigations. Multiple methods of PD immobilisation have been attempted to date and their performance evaluated, including: (i) Gink doping at various loadings (ii) electrodeposition of a PD poly/oligomer film on the underlying GInk and (iii) enzymatic PD polymerisation in the presence of glucose oxidase, aided by substrate addition and subsequent hydrogen peroxide production. A "layer by layer" enzyme entrapment method was employed for glucose oxidase entrapment and the electrocatalytic response upon glucose additions was followed using cyclic voltammetry and differential pulse voltammetry (DPV) resulting in calibration curves over the range 0.1-2.0 mM glucose. The optimum method (iii) was transferred as a proof of principle to a carbon cloth electrode (Fig. 2) and successful glucose response was obtained as a result of monitoring the electrocatalytic cathodic DPV process.



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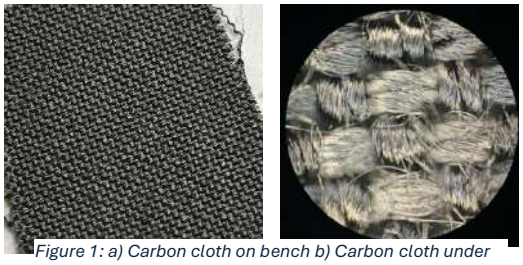

Figure 1: a) Carbon cloth on bench b) Carbon cloth under reflectance microscope x10 mag

PO 05:

LIQUID CHROMATOGRAPHY COUPLED WITH AMPEROMETRIC DETECTION TO ASSESS THE NUTRITIONAL VALUE AND THERMAL DAMAGE OF GLUTEN-FREE PASTA

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In recent years, the growing number of people diagnosed with coeliac disease or gluten intolerance and/or who have special dietary habits has led to an increased demand for gluten-free products. Among gluten-free foods, pasta is one of the most commonly consumed due to its convenience, palatability and long shelf life.
Drying pasta is a fundamental step to reduce the moisture content of pasta and extend shelf life by reducing water activity and inactivating enzymes. During this process, however, the Maillard reaction and thus «thermal damage» can occur. The so-called non-enzymatic browning starts with the condensation between a primary amino group of amino acids and proteins and a carbonyl group of a reducing sugar and then involves a cascade of reactions that strictly depend on the initial composition of the food matrix, the extent of heat treatment applied, water activity, pH and moisture during the food manufacturing process.
In the present study, liquid chromatography was used in conjunction with amperometric detection to assess the content of amino acids and sugars in gluten-free commercial pasta samples.
Among the compounds that can be used as markers for the drying process, the descriptors of the early (furosine) and advanced stages (hydroxymethylfurfural and glucosylisomaltol) of the Maillard reaction were detected.

PO 06:

SCALABLE AND COST-EFFECTIVE IN SITU LASER SYNTHESIS OF NANOZYMES: PIONEERING THE FUTURE OF ENZYME-BASED ELECTROCHEMICAL SENSORS

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This work introduces a novel and scalable in situ laser-assisted approach for synthesizing nanozyme materials, aimed at advancing the development of enzyme-based electrochemical sensors. Using UV laser diodes at varying wavelengths, we successfully engraved graphene doped with various sorts of magnetic nanoparticles (MNPs). Comparative electrochemical analysis revealed that the laser-engraved graphene-MNPs composite significantly enhanced electron transfer rates, resulting in increased sensitivity and selectivity for target analytes. The integration of magnetic nanoparticles also conferred superior stability and resistance to fouling, further supporting its applicability in real-world sensor platforms. Additionally, the tunability of the laser wavelength allowed for precise control over the synthesis process, offering a cost-effective and scalable pathway for nanozymes production, creating a hybrid material with enhanced catalytic properties. The resulting nanozymes, mimicking the activity of natural enzymes, mainly laccase and peroxidase, demonstrated promising catalytic efficiency in detecting hydrogen peroxide and phenolic substrates, including dopamine and caffeic acid. In summary, this scalable in situ laser synthesis method presents a powerful alternative to conventional enzyme immobilization strategies, opening new horizons for next-generation electrochemical sensors with superior performance in detecting key biomolecules and environmental contaminants.

Keywords: Laser assisted synthesis, Nanozymes, Magnetic nanoparticles, Peroxidase and laccase activity, Electrochemical sensors.

PO 07:

POLY(E-CAPROLACTONE) (PCL) NANOENCAPSULATED METRIBUZIN OPTICAL DETECTION USING THE GREEN PHOTOSYNTHETIC MICROALGA CHLAMYDOMONAS REINHARDTII

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Agriculture is a major cause of environmental pollution worldwide, as agricultural activities have been oriented towards intensive practices, including the indiscriminate use of pesticides that contribute to soil and water contamination. This has major consequences for ecosystems and human health, so that defined maximum residue levels (MRLs) of these chemicals in food and water have been established to limit their use (Drinking Water Directive 0.1-0.5 μg/L, CD 98/83/EC) [1,2].

Consequently, more sustainable agriculture and waste minimization have become imperative. In this context, smart agriculture implies the exploitation of crosscutting approaches based on energy-efficient and environmentally friendly technologies, including i) novel nanoformulations to increase dispersion and wettability of pesticides and ii) advanced diagnostic tools for water analysis [3].

In recent years, diverse biosensors have been designed for the detection of nanoformulated herbicides (i.e. atrazine as case study), using the green photosynthetic microalga Chlamydomonas reinhardtii as biological recognition element in different configurations:

an optical paper-based biosensor for the detection of nanoencapsulated atrazine in tap water with a very high sensitivity up to 4 pM [4];

an all green electrochemical biosensor to reveal nanoformulated atrazine in wastewater with a detection limit of 0.9 and 1.1 nM for atrazine-zein and atrazine-PCL-Chitosan, respectively [5].

In the present study, we designed a bioassay for the detection of a novel nanoherbicide, i.e. metribuzin entrapped into nanocapsules of poly(ε-caprolactone) (M-PCL), specifically developed for more efficiency gains through targeted delivery and environmental risk reduction [6]. In particular, the C. reinhardtii CC125 strain was cultivated to reach an exponential phase of the grow curve (0.9 O.D. at 750 nm wavelength) and incubated under illuminating light (50 μmol photons/m2/s) at room temperature (25°C) for 30 min with increasing concentrations of M-PCL (1-100 nM). After that, the microalgal incubated with M-PCL was kept in dark for 10 min and Kautsky profiles were recorded to highlight the effect of the nanometribuzin on the photosynthetic parameters, i.e. the variable fluorescence 1-VJ, which provide a specific response correlated to the herbicide-D1 binding niche interaction [7]. A linear range was obtained between 1 and 100 nM with a detection limit of 4.16 pM, calculated as $2.6 \times \sigma \times I20/100 - 2.6 \times \sigma$, where σ is the standard deviation of the response. Further studies will be devoted to the evaluation of matrix and interferents effects on the analytical performances of the biosensor.

PO 08:

Evaluation of the Applicability of an ELISA Kit for the Determination of WGA in Milled Fractions Produced During the Milling Process of Durum Wheat

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Wheat germ agglutinin (WGA) is a dimeric protein with a molecular weight of 36 kDa that belongs to the lectins. It occurs exclusively in the germ and has, therefore, been chosen as a unique protein marker for whole wheat.
The aim of this work is to test and evaluate the applicability of a commercial ELISA kit for the determination of WGA content in products from the milling process of Triticum durum. The identification of tissue-specific compounds, such as WGA, whose concentration measured in mill streams is directly linked to the proportion of germ, will be useful to reveal and quantify the presence of wheat germ in industrially (re-)combined flours.
The commercial kit is an ELISA in which WGA (standard solutions or unknown samples) and WGA-biotin compete for the specific antibodies coated onto the plate. Subsequently, the streptavidin-HRP conjugate binds biotin with a strong and stable interaction. Finally, the HRP substrate is added, and the intensity of the colorimetric signal is inversely proportional to the amount of WGA in standard solutions or samples.
After optimization of some parameters concerning the extraction procedure of WGA (i.e. ratio gsample/VRIPA buffer, sonication cycles) and the appropriate dilution of the lysate before the analysis, the milled fractions of Triticum durum were processed and analyzed. The results obtained, in terms of WGA concentration, were in line with the type of fractions produced during the milling process.
However, in most cases, the concentration values were poorly repeatable (RSD up to 36%), even though the recorded signals showed an RSD $\leq$ 13%. This can be ascribed to the low sensitivity of the commercial kit: poor slope of the calibration straight line in a wide range of concentrations (9.7-10000 ng/mL). To overcome this limitation, the development of an ELISA method with greater sensitivity and lower cost is under study.

PO 09:
REAGENT-FREE PHYTIC ACID ELECTROCHEMICAL DETECTION IN SPINACH USING A 3D-PRINTED AND PAPER-BASED BIOSENSOR

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Phytic acid is a phosphorylated derivative of myo-inositol, widely present in plants as their primary phosphorus storage form. Although beneficial for plants, it's considered an antinutrient in human diets. By binding essential minerals like calcium, iron, and zinc, phytic acid reduces their bioavailability, highlighting the importance of its monitoring in food products [1]. In this scene, we have developed a reagent free paper-based biosensor for quantifying phytic acid in spinach leaves integrated in a 3D printed extraction device. Phytase, immobilized on screen-printed electrodes modified with carbon black dispersion, hydrolyzes phytic acid generating phosphate ions that form an electroactive complex with the molybdate pre-loaded on a filter paper pad [2]. The oxidation of phosphomolybdate complex was monitored by using cyclic voltammetry. A linear response was observed across a phytic acid concentration range of 1 to 50 µM with a limit of detection of 0.8 µM in standard solutions. To simplify phytic acid analysis in spinach leaves, a 3D printed funnel was attached to a grinder to directly channel the extracted solution onto a paper electrode. Spinach leaves samples were ground and treated with 0.2 M HCl within the 3D printed device. Developing biosensor for phytic acid detection in foods is crucial for enhancing nutritional quality, ensuring food safety, and mitigating health risks.

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PO 10:
FIBER-OPTIC SENSORS FOR ANTIBODIES DETECTION

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Sensors play a key role in modern medicine by enabling precise monitoring and measurement of physiological parameters. From wearable devices to sophisticated imaging equipment, sensors can provide real-time data that is crucial for diagnosis, treatment, and further patient care [1]. Fiber optic sensors have great potential for medical measurements as they offer advantages such as high sensitivity, immunity to electromagnetic interferences, compact size, and the capability for remote sensing [2].
In this research, we designed, developed and validated the fiber-optic sensor to detect biomarkers in the sample. The biofunctionalized surface of the fiber tip allows only attachment of the molecules of interest, for example proteins [3] or antibodies [4]. The principle of the operation is based on interferometry: depending on the presence and level of measured substances, the optical parameters of the layer change due to their attachment to its surface, which leads to a change in the reflected optical signal.
The measurement system construction includes a light source, optical spectrum analyzer and 2x1 fiber coupler with biofunctionalized fiber tip. We present the results of two investigations including measurements of different agents: CRP, one of the most important biomarkers used to determine inflammation presence in the body, and the SARS-CoV-2 specific IgG antibodies. We have shown that the sensors' parameters are sufficient for medical purposes, offering short measurement-to-result time (<10 min), need for small sample amount, user-friendly operation and the use of commercially available components.

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PO 11:
AN INGENIOUS PLATFORM FOR NUCLEIC ACID IMMOBILIZATION BASED ON POLYSTYRENE 96-WELL MICROPLATE HYDROXYLATION

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Genosensors have emerged as valuable tools for detecting biomolecules across various sectors like agriculture, environmental monitoring, and healthcare. Particularly, DNA genosensors are gaining traction due to their sensitivity, cost-effectiveness, and potential for miniaturization, making them ideal for detecting biomarkers such as miRNAs, which are implicated in diseases like cancer.

Constructing a DNA biosensor involves several key steps: preparing the immobilization platform, immobilizing the probe, recognizing miRNAs through hybridization with the probe, and detecting biological recognition. The choice of immobilization platform is critical for biosensor development. miRNA-222, a promising target for early cancer detection, was selected for this study, offering significant potential for improved diagnosis and treatment.

A new approach was employed to covalently immobilize probe DNA onto polystyrene microplates modified with hydroxyl groups, using glutaraldehyde as a cross-linking agent. The use of 96-well polystyrene microplates, a simple, cost-effective, and multiplexing platform for DNA immobilization and miRNA/DNA detection, provides reassurance in its practicality. The recognition probe hybrids were detected using an alkaline phosphatase (ALP) with avidin. The presence of the target miRNA was evaluated through a colorimetric assay using para-nitrophenyl phosphate (PNPP) substrate, which produces a yellow color measurable at 405 nm after enzymatic conversion. The colorimetric genosensor assay demonstrated a linear detection range from 0.5 nM to 20 nM for miRNA-222, with a detection limit of 0.3 nM.

Keywords: Immobilization platform, DNA biosensors, miRNAs, Colorimetric detection, 96-well polystyrene microplates.

PO 12:

DEVELOPMENT OF A NEW NANOBIOPLATFORM BASED ON GREEN GOLD SONONANOPARTICLES FOR THE SIMULTANEOUS DETECTION OF DOPAMINE (DA) AND SEROTONIN (5-HT)

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In this work, a Sonogel-Carbon electrode (SNGCE) modified with pine leaves extract-derived gold sononanoparticles (AuSNPs) is reported for the first time, for the simultaneous detection of serotonin (5-hydroxytryptophane, 5-HT) and dopamine (DA) as critical influencers in the human health care system. The biosynthesized AuSNPs were characterized using different optical, structural and morphological techniques including UV-VIS, DLS, FTIR and SEM techniques. The formation of AuSNPs on the SNGCE surface was confirmed by the presence of an adsorption band at 538 nm. This was further confirmed by the white specks at the SEM micrographs indicating the presence of dispersed nanoparticles with an average size of 4 ± 2 nm obtained from DLS analysis. The experimental parameters affecting the sensor performances were optimized in terms of AuSNPs coating amount, scan rate and electrolyte pH in the range 3 - 9. The electrochemical performances of the developed sensor were evaluated using DPV technique. The simultaneous detection of both analytes indicates an excellent limit of detection (LOD) of 750 pM and 530 pM with a detection range from 1 to 20µM for 5-HT and DA respectively. Furthermore, the sensor shows good selectivity for both 5-HT and DA when several interferents were introduced, with a relative standard deviation (RSD) of about 4 %. More importantly, the simultaneous detection of both 5-HT and DA neurotransmitters were evaluated in human blood serum and demonstrated an excellent recovery rate for 5-HT and DA. The main advantages of the designed sensor are its simplicity, portability, and low cost revealing a strong potential for application in several fields including medical diagnosis in a sustainable and economic route.
Keywords: Pine leaves extract; electrochemical sensor; Sonogel-Carbon electrode; dopamine; serotonin; gold sononanoparticles; simultaneous detection.

PO 13:

PERFORMANCE EVALUATION OF AN OPTIMIZED HIGHLY SENSITIVE PCF-SPR BIOSENSOR FOR CANCER CELLS DETECTION

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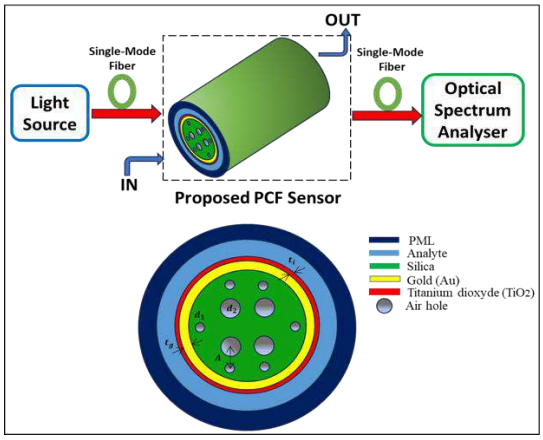
In recent years, surface plasmon resonance (SPR) based photonic crystal fiber (PCF) sensors have seen significant advancements. However, many existing designs suffer from low sensitivity or complex fabrication processes that are not suitable for real-time applications. We present a novel, easily fabricated circular PCF-SPR biosensor designed for the early detection of cancer cells, with high sensitivity across visible and near-infrared spectra. This biosensor features an external gold (Au) coating as the plasmonic layer, enhanced by a thin titanium dioxide (TiO2) layer to improve adhesion between the gold and the PCF surface. By optimizing five key design parameters, including air hole diameters, pitch, and the thicknesses of the TiO2 and gold layers, using the Taguchi L8(25) orthogonal array method, we achieved exceptional spectral sensitivity of 18000 nm/RIU and amplitude sensitivity of 681.655 RIU-1. The biosensor's broad refractive index (RI) detection range (1.29-1.40) makes it suitable for detecting cervical, blood, and skin cancer cells. Notably, the biosensor demonstrates a peak sensitivity of 7500 nm/RIU for cervical cells and amplitude sensitivity of 773.466 RIU-1 for skin cells. Additionally, a Multi-Layer Perceptron Artificial Neural Network (MLP-ANN) model was employed to predict the sensor's performance. The findings demonstrate the efficiency of artificial neural networks in providing quick and accurate predictions for various geometric configurations, showcasing their potential in improving advanced biosensor applications.
Keywords: Surface Plasmon Resonance, Photonic Crystal Fiber, Taguchi Approach, Cancer detection, Artificial Neural Network.



PO 14:
PROTEIN-BASED FIBERS FOR FLUORESCENT BIOSENSORS

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The use of eco-friendly and easy functionalization procedures is gaining interest in developing sensing platforms in different sectors. For this purpose, Hydrophobins (HPBs) offer a new and very valuable alternative for surface functionalization. HPBs are small fungal surface-active proteins, able to self-assemble into amyloid fibrils and adhere to several surfaces. Fibrils formed by Vmh2, an HPB from the fungus *Pleurotus ostreatus,* display intrinsic fluorescence, a relatively new and not completely understood phenomenon, possibly related to a partial delocalization of π-electrons of peptide bonds along the extended structure typical of amyloid fibrils [1]. This phenomenon has been exploited to detect Hg (II) by the chimeric protein that combines Vmh2 and the Hg (II) binding peptide (H3W). The autofluorescence of amyloid fibrils was quenched by increasing Hg (II) concentration, allowing the detection of Hg (II) in the nM range, also in the presence of other metals [2].
Moreover, it has been observed that autofluorescence emission is pH-sensitive, the lower the pH the lower the emission. This correlation could be exploited to develop other interesting sensing devices, useful for intelligent and smart packaging materials.

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PO 15:
PAPER-BASED ELECTROCHEMICAL (BIO)SENSORS FOR A DISRUPTIVE ORIGAMI ORGAN-ON-CHIP DEVICE: PHOENIX-OOC

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In recent years, organ-on-chip (OoC) technology has emerged as a revolutionary approach in the field of biomedical research, offering significant advancements over traditional in vitro and in vivo models. These microengineered devices replicate human organs' complex physiological and mechanical properties on a micro-scale, providing a more accurate and reliable platform for studying human biology and disease. Generally, OoC devices consist of microfluidic systems that integrate living cells cultured in a 3D environment, mimicking the architecture and function of specific human organs. This biomimetic approach allows for the recreation of dynamic biological processes, including tissue-tissue interfaces, fluid flow, and mechanical forces, which are critical for organ function.1 Sustainability is one of the most important concepts today, as it can drive activities in several areas, namely environmental, social, and economic. In analytical chemistry, the development of sustainable devices has been boosted by the introduction of paper-based microfluidic analytical devices (μPADs), whose benefits are not limited to the concept of sustainability. Indeed, paper as a functional material gives μPADs unprecedented properties. However, paper-based devices are still used only as analytical tools and have not been adopted by the OoC world. In this context, we present a revolutionary OoC paper-based platform that uses paper in origami configuration for i) cell co-cultures with the aim to better simulate different organ tissues, (ii) (bio)sensors integration with the aim of on site/continuous monitoring of cells status/response to stimuli, and (iii) with the ultimate goal of performing accurate pharmacological studies. In detail, the OoC device includes a paper-based scaffold consisting of a cage printed using a 3D technique, containing the cell pads in origami configurations preloaded with the different cell lines, the electrochemical sensors modified for the detection of several analytes, and the adsorbent pad as waste. In the first part of the project, electrochemical sensors for pH, nitrate, and glucose were developed and characterized in standard solutions. The sensors were screen printed onto a filter paper support using wax to create a hydrophilic zone delimited by hydrophobic wax barriers. To create layers sensitive to the different ions, the working electrode surface was modified with nanomaterial and ion-selective membranes for nitrate detection, while for H+ detection the surface was modified with a layer sensitive to pH variations. For glucose monitoring, the sensor was modified by drop casting with nanoparticle dispersion and glucose oxidase enzyme.

Calibration curves were obtained by potentiometric measurements in standard solutions with regression equations $y= (-0.048\pm0.002)\ x + (0.359\pm0.004)$; $R^2 =0.981$, $y= (-0.083\pm0.001)\ x + (0.763\pm0.\ 007)$; $R^2=0.998$, for nitrate and pH respectively, and by chronoamperometric technique for glucose detection, a linear regression described by the following equations was obtained: $y= (-0.12\pm0.01)\ x - (0.7\pm0.1)$; $R^2 =0.960$.

The development and application of OoC systems hold great promise for improving drug discovery, reducing animal testing, and advancing our understanding of human physiology and pathology. As this technology continues to evolve, it is expected to play a critical role in the future of medical research and personalized healthcare.

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PO 16

The Mass Production of Molecularly Imprinted Polymers via Cost-Effective Photopolymerization synthesis and Colorimetric detection via smartphone

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This work presents an innovative approach for the rapid synthesis and mass production of Lab-on-Paper devices integrated with molecularly imprinted polymers (MIPs) via cost-effective in-situ photopolymerization, enabling one-step, on-site detection of sulfamethoxazole (SMX). Conventional methods for fabricating paper-based analytical devices often face challenges related to scalability, reproducibility, and sensitivity. To address these limitations, a streamlined process was developed, allowing for efficient production of paper-based devices. The platform enhances detection capabilities, particularly in the analysis of SMX, a widely used antibiotic with significant environmental impact. The integration of MIPs synthesized via photopolymerization ensures precise molecular recognition, improving sensitivity and accuracy compared to traditional methods. The cost-effective nature of in-situ photopolymerization not only reduces production costs but also facilitates large-scale manufacturing. The user-friendly platform offers practical applications in various fields, including environmental monitoring, food safety, and healthcare. The proposed approach is based on smartphone-readout colorimetric detection, utilizing the complexation of SMX with selective binding agents directly on the paper substrate. This significantly enhances the sensitivity and applicability of the Lab-on-Paper devices for SMX detection, providing a portable and convenient solution for on-site analysis. Overall, this study highlights the potential of this method to revolutionize the development of cost-effective and accessible analytical tools for SMX detection and beyond, driving advancements in paper-based sensing technologies.

Keywords: Lab-on-paper devices, molecularly imprinted polymers, sulfamethoxazole, in-situ photopolymerization, mass production

PO 17:
ADVANCING BIOMEDICAL SENSING: DIAMOND STRUCTURES FOR FIBER-OPTIC SENSORS

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Sensors have become an integral part of today's world due to their ability of delivering precise data about variety of parameters on-demand or in real-time. As such, they found applications in eg, environment monitoring, automotive and automation industries, agriculture. They are also transforming healthcare through vital signals tracking, detection of biomarkers, measuring of specified agents levels, leading to earlier diseases detection and diagnosis support [1]. Optical sensors deliver contactless, non-invasive operating mode, high sensitivity and the need for minimal sample amount. Moreover, with fiber-optic engagement, such sensors are small and robust, allow integration with existing telecommunication systems and are immune to electromagnetic interferences [2].
In our research, we integrated fiber optic sensors with diamond structures, leveraging the possibilities of their synergy. Diamond materials offer chemical stability, biocompatibility, and the structure parameters can be tailored to specific requirements [3]. They are also known for their hardness, which enhances the durability of the sensors elements, making them ideal for demanding biomedical applications [4,5].
Our work presents the design and development of an interferometric optical fiber sensor. The setup is built of the superluminescence diode, optical spectrum analyzer and 2x1 fiber coupler. The process of diamond structure deposition and their role in the system is described. We also present the measurement procedure, results and data analysis of the optical spectra on the example of measuring the hemoglobin level of patients' blood. The developed sensor presents a linear work characteristics and a correlation coefficient (R2) of 0.988.

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PO 18:

RAPID PHOTOSYNTHESIS OF A MOLECULARLY IMPRINTED MEMBRANE FOR SELECTIVE COLORIMETRIC ANALYSIS OF AN ANTIBIOTIC

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The accurate detection of antibiotics, which serve as vital synthetic antimicrobials, is essential for safeguarding public health. This study introduces a groundbreaking methodology for the creation of a molecularly imprinted membrane (MIM) sensor, which utilizes biopolymers as the matrix material and the antibiotic as an analyte. The synthesis of the MIM occurs rapidly within a mere 5 minutes via UV-assisted photopolymerization, significantly enhancing both the speed and efficiency compared to conventional methods. The integration of biopolymers confers exceptional stability to the membrane in organic solvents, alongside outstanding mechanical flexibility and rigidity. This advanced biopolymer, noted for its superior tensile strength and structural integrity, enhances the performance and longevity of the MIM, rendering it a highly dependable material for high-performance sensing applications.

Following the MIM synthesis, a range of characterization techniques such as X-ray diffraction, thermogravimetric analysis, Fourier-transform infrared spectroscopy, and mechanical testing were employed to evaluate the semicrystalline, thermal, structural, and mechanical properties, thereby confirming the MIM's high quality and effective performance for sensing applications. Additionally, a rapid, straightforward, reproducible, and highly sensitive colorimetric method for antibiotic detection has been developed, further complementing the MIM sensor's capabilities. The MIM sensor exhibited a notable limit of detection (LOD) of 0.03 µg/mL and a limit of quantification (LOQ) of 0.1 µg/mL, demonstrating its capability to detect trace amounts of the antibiotic (0.16 ng/mL) through preconcentration via solid-phase extraction (SPE). Validation of the methodology in real-world contexts, including spiked river water and saliva samples, revealed excellent recovery rates ranging from 94.21% to 100%. The MIM-based sensor marks a significant advancement in the realm of on-site colorimetric analysis, providing a practical, high-performance, and portable solution for real-time monitoring.

Keywords: Molecularly imprinted membrane; antibiotic; biopolymers; UV-assisted photopolymerization; colorimetric detection; solid-phase extraction.

PO 19:
FAST SYNTHESIS OF MOLECULARLY IMPRINTED POLYMERS FOR SELECTIVE EXTRACTION OF PHYTOSTEROLS IN FOOD SAMPLES

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Phytosterols (PSs) are bioactive compounds structurally and functionally similar to cholesterol. They contain an extra methyl, ethyl group, or double bond, and most of their side chains contain 9-10 carbon atoms. PSs have been classified as 4-desmethyl sterols of the cholestan series, which all have double bonds at the C5 position of the B-ring1. These molecules are particularly known for widely range of properties including reduced intestinal cholesterol absorption and potential contributions to the prevention of cardiovascular diseases2. PSs are generally classified into three groups based on the number of methyl groups on carbon-4, two (4-dimethyl), one (4-monomethyl), or none (4-desmethyl). Moreover, 4-dimethyl esters and 4-monomethylsterols are metabolic intermediates in the biosynthetic pathway leading to the final product, 4-desmethyl phytosterols, but are usually present at low levels in most plant tissues. These compounds are present in plants, such as seeds, grains and legumes, both in free and conjugated form and they can be found in the form of fatty acyl esters, glycosides and fatty acyl glycosides. The selectivity issue in plant matrices is a challenging task, and classic approach such as solvent or solid phase extraction (SPE) are expensive and not always give the needed selectivity. In this scenario a low cost molecularly imprinted polymer (MIP) approach for the selective of extraction these compounds was not fully explored. In this work, a fast chemical MIP synthesis approach for selective extraction of PSs was performed, taking advantage of a high-power ultrasound-prob using metacrilic acid as functional monomer, ethylene glycol dimethacrylate as cross-linker, AIBN as initiator, and cholesterol as a dummy template. The MIPs were used as an adsorbent phase in dispersion SPE and combined with a targeted approach using liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) with atmospheric pressure chemical ionization (APCI). The sorbent ability of MIPs was proved towards PSs extraction in model solutions, demonstrating useful binding ability and outstanding selectivity, allowing PSs extraction in polar solutions. The results of application of MIP-dSPE in different real samples showed high selectivity with significant extraction performances (60-100%) and low matrix effect (<15%), a principal goal in complex plant matrices. The proposed strategy can be considering as fast and effective method to produced MIPs as extraction tool for the determination of these target compounds.

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PO 20:

MICROALGAE BIOTECHNOLOGY: APPLICATIONS OF THE GREEN PHOTOSYNTHETIC ALGA CHLAMYDOMONAS REINHARDTII FROM BIOSENSING TO BIOFARM AND BIOREMEDIATION

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Microalgae have been widely exploited in biosensing development, as they have demonstrated considerable potential for sensitive, sustainable, and multiplexed detection of analytes of agro-environmental and security interest. Their advantages include the availability of diverse algal bioreceptors, including whole cells and their photosynthetic subcomponents, their potential to be integrated into miniaturized dual-transduction devices, and the opportunity for continuous monitoring1. Despite obstacles, including limited stability and selectivity, microalgae biosensors are a realistic prospect with effective applications. Several biosensors have been described in the literature using the green photosynthetic microalga Chlamydomonas reinhardtii immobilized on divers supports (e.g. paper, screen-printed electrodes), modified with different nanomaterials (e.g. quantum dots, carbon black), integrated into dual optical-electrochemical transductions, for the detection of a wide range of chemical including herbicides and chemical warfare agents simulants2-8.
In addition to their use in biosensing design, biomass production and bioremediation are alternative biotechnological applications. Numerous studies have underpinned the potential of C. reinhardtii as biochemical factory, capable of producing various molecules with a direct impact on human health and longevity. Polysaccharides, lipids, proteins, pigments, hormones, vaccines, and antibodies are among the valuable biomolecules that are produced by microalgae and can be directly linked to human nutrition and diet9. On the other hand, bioremediation using C. reinhardtii presents significant potential for sustainable reduction of environmental contaminants10, while facilitating resource recovery and valorization of microalgal biomass for the production of added-value compounds.

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PO 21:
HYDROGEN PEROXIDE MONITORING IN EXHALED BREATH BY AN INNOVATIVE ALL-PAPER-BASED SENSING PLATFORM

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Point-of-care sensing devices play a crucial role in assisting clinicians with quick, analyses, easy management, and painless monitoring of patient diseases. These sensing tools closely align with the ASSURED criteria set by the World Health Organization, where ASSURED stands for affordable, sensitive, specific, user-friendly, rapid, equipment-free, and deliverable to end-users. This concept was further updated to REASSURED, emphasizing real-time connectivity and ease of specimen collection.

In this context, in the past ten years, there has been significant progress in creating new point-of-care (POC) sensors that exploit the various properties of paper, such as porosity, capillary forces, and ease of modification. Indeed, paper has become a focus for developing electrochemical POC sensors due to its many advantages, including i) not requiring external equipment for liquid flow, ii) the ability to create reagent-free devices, and iii) the ease of performing multistep analyses using origami techniques. Additionally, an important aspect of using paper is its capacity to hold reagents needed for analysis. This enables the paper-based sensor to be exposed to aerosols, such as breath, allowing the paper to become wet and dissolve the stored reagents. As a result, exhaled breath aerosol, can be used as a matrix for analysis, making it easy to collect by incorporating collecting paper into a face mask.

In this overall context, we are developing a whole-paper-based analytical tool for hydrogen peroxide detection in exhaled breath, taking into account the high level of H_2O_2 in lung-associated diseases.

In this work, we leverage the use of an office paper electrochemical screen-printed electrode, and a flower-like paper collector integrated into a face mask. Specifically, the paper-based collector enables the non-invasive sampling and the further treatment-free analysis of the biological sample. The screen-printed electrode is modified with a carbon black-Prussian Blue nanocomposite, enabling reliable detection of hydrogen peroxide in the condensed aerosol breath sample.

We are currently investigating the applicability of the flower-shaped paper collector, considering factors such as size, thickness, and shape. Additionally, we are optimizing the screen-printed sensor to improve parameters like linearity range, limit of detection, selectivity, and accuracy.

In conclusion, the proposed analytical platform has the potential to open up new possibilities for the design of face masks as active analytical platforms for breath analysis, aiming to facilitate health management and improve the quality of patient life.

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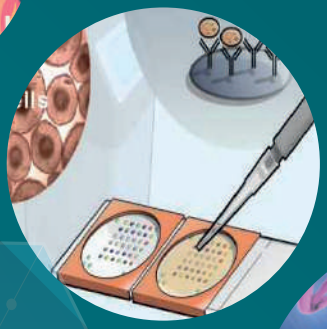

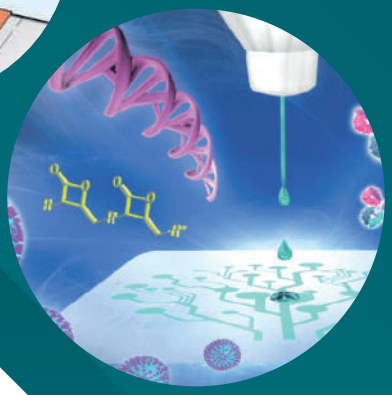

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