LIBRO DE RESÚMENES

III REUNIÓN DEL GRUPO ESPECIALIZADO EN CIENCIA Y TECNOLOGÍAS (BIO)ANALÍTICAS DE LA RSEQ

Bilbao-Sarriko, 4 de julio de 2025

Grupo de Ciencia y Tecnologías (Bio)Analíticas

Libro de Resúmenes

III Reunión Científica del Grupo Especializado de Ciencia y Tecnologías (Bio)Analíticas de la RSEQ

Bilbao-Sarriko, 4 de julio de 2025

Libro de Resúmenes de la III Reunión Científica del Grupo Especializado de Ciencia y Tecnologías (Bio)Analíticas de la RSEQ.

Jose Manuel Costa Fernández, Alfredo de la Escosura Muñiz y Ramón J. Barrio Diez-Caballero (Editores)

Grupo de Ciencia y Tecnologías (Bio)Analíticas

GRUPO METABOLOMIPs

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Bienvenida de la Junta de Gobierno a la III Reunión Científica del GCTbA-RSEQ

Queridas y queridos colegas:

En nombre de la Junta de Gobierno del Grupo Especializado de Ciencia y Tecnologías (Bio)Analíticas de la Real Sociedad Española de Química (GCTbA-RSEQ), es un placer daros la bienvenida a la **III Reunión Científica del GCTbA-RSEQ**, un encuentro que consolida el camino iniciado en Granada en 2022 y continuado en Zaragoza en 2023.

Desde la Junta queremos expresar nuestro más sincero agradecimiento por vuestra implicación, entusiasmo y compromiso con este joven grupo, que se encuentra ya en plena fase de consolidación. Vuestra participación fue el motor que impulsó, en abril de 2020, la creación del GCTbA-RSEQ, que hoy cuenta con 290 socios y socias unidos por el firme propósito de fortalecer la presencia de la Química Analítica dentro de la RSEQ —una de las sociedades científicas más relevantes de España—, contribuyendo desde una perspectiva transversal, integradora y colaborativa.

Una vez más, vuestra respuesta ha sido excepcional. En esta edición hemos registrado cerca de 120 inscripciones y recibido aproximadamente 90 resúmenes científicos. El comité científico ha trabajado intensamente en la elaboración de un programa que refleje la diversidad de líneas de investigación desarrolladas en nuestro país.

Las comunicaciones orales tendrán una duración de 10 minutos, lo que permitirá incluir un mayor número de contribuciones y ofrecer una visión lo más amplia posible de la actividad en química (bio)analítica a nivel nacional. Además, se ha reservado un amplio margen horario para facilitar y potenciar la visita a los pósteres, con el objetivo de fomentar la interacción entre los asistentes. Cabe destacar que, en coherencia con nuestro compromiso con los jóvenes investigadores, las presentaciones orales estarán a cargo de doctorandos y jóvenes investigadores postdoctorales.

El resultado es un programa científico de gran calidad, que incluye tres conferencias invitadas a cargo de los galardonados con los Premios GCTbA-RSEQ a las Mejores Tesis Doctorales en el ámbito de la Ciencia y Tecnologías (Bio)Analíticas (ediciones 2023 y 2024), así como del ganador del concurso “Tu investigación en 3 minutos” (edición 2024). A estas se suman 27 presentaciones orales y 59 comunicaciones tipo póster.

Sabemos que la Química Analítica es hoy, más que nunca, una herramienta clave para afrontar desafíos en ámbitos tan diversos como la salud, la sostenibilidad, el medioambiente, la energía, los materiales, así como el control de la calidad y la seguridad alimentaria. Su vocación por la transferencia de conocimiento forma parte esencial de nuestra labor diaria, y así se refleja en el valioso apoyo de empresas como Agilent Technologies, Biolan, i+Med, Lasing y Scharlab, a quienes agradecemos muy sinceramente su colaboración. También queremos expresar nuestro agradecimiento a las revistas *Microchimica Acta* (Springer Nature) y *Analytical & Bioanalytical Chemistry* (Springer Nature) por su apoyo mediante el patrocinio de premios a las mejores contribuciones científicas, así como a la Universidad del País Vasco, cuyo respaldo institucional y generosa cesión de espacios han hecho posible celebrar esta reunión en un entorno tan acogedor.

Durante el encuentro celebraremos también la III Asamblea General de Socios del GCTbA-RSEQ, en la que se hará balance de las actividades desarrolladas en los últimos dos años y se presentarán las iniciativas

previstas para los próximos meses. Asimismo, se hará entrega de los galardones correspondientes a la tercera y cuarta edición de los Premios GCTbA-RSEQ a la Mejor Tesis Doctoral en el ámbito de la Ciencia y Tecnologías (Bio)Analíticas, así como el de la segunda edición del concurso "Tu investigación en 3 minutos". Nuestra más cálida enhorabuena a las personas distinguidas con estos reconocimientos.

No queremos concluir sin expresar nuestro más profundo y entusiasta agradecimiento al Profesor Ramón Barrio, presidente del comité organizador del Worshop, cuya implicación, liderazgo y generosidad han sido fundamentales para el éxito de esta reunión. Su dedicación, junto con la de su equipo de investigación, ha permitido cuidar cada detalle de la organización, brindándonos la oportunidad de disfrutar de un encuentro verdaderamente excepcional. Muchas gracias, Ramón, por todo el esfuerzo realizado para hacer posible este evento.

Gracias, una vez más, a todas y todos por hacer posible este proyecto colectivo. Os deseamos una estancia inspiradora en Bilbao, y que esta III Reunión Científica sea un espacio fértil para el intercambio de ideas, la creación de sinergias y el impulso de nuevas iniciativas que sigan enriqueciendo nuestra disciplina.

Con afecto y gratitud,

José Manuel Costa Fernández
Presidente del GCTbA-RSEQ

Bienvenida del Comité Organizador

Estimadas y estimados participantes en la GCTbA-2025:

Desde el Comité Organizador queremos daros la más cordial bienvenida a la III Reunión del Grupo Especializado de Ciencia y Tecnologías (Bio)Analíticas de la RSEQ (GCTbA-2025).

Nuestro objetivo ha sido siempre crear un espacio agradable de intercambio de conocimientos, ideas y experiencias que enriquezcan a todos los asistentes. Esperamos que disfrutéis de las actividades que se han organizado y que podáis aprovechar al máximo esta oportunidad de colaboración entre todos los miembros de nuestra comunidad BioAnalítica.

Agradecemos sinceramente la participación de las empresas patrocinadoras y muy especialmente a la Facultad de Economía y Empresa de la UPV/EHU, por todas las facilidades que nos ha dado para celebrar esta III Reunión del GCTbA en sus magníficas instalaciones.

Estamos seguros de que disfrutaréis de vuestra estancia en Bilbao.

¡ Bienvenidos, *Ongi etorri* !

Ramón J. Barrio

Presidente del Comité Organizador

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GRUPO METABOLOMIPs

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Programa Científico

Viernes 4 de julio de 2025

8.30-9.00 Acreditación, entrega de documentación y colocación de pósters

9.00-9.20 INAUGURACIÓN DE LA III REUNIÓN DEL GCTbA y Entrega Premios .
Moderadores: Jose Manuel Costa y Ramón J. Barrio

9.20-9.35 ***Conferencia Oral Invitada****: Premio Tesis 2023:* ***Eloy Povedano****. BIOELECTROANALYTICAL PLATFORMS WITH MULTIPLEXING AND MULTI-OMICS POTENTIAL FOR PRECISION MEDICINE AT POINT OF CARE*

9.35-9.50 ***Conferencia Oral Invitada****: Premio Tesis 2024:* ***Alberto Celma****. THE ROLE OF ION MOBILITY-HIGH RESOLUTION MASS SPECTROMETRY FOR THE ANALYSIS OF ORGANIC MICROPOLLUTANTS IN COMPLEX MATRICES*

9.50-10.00 ***Conferencia Oral Invitada****: Premio Investigación en 3 min 2024:* ***Víctor Pérez****. INSTALLING CAMERAS IN THE FACTORY: ELECTROCHEMICAL BIOSENSORS FOR EARLY MOLECULAR SURVEILLANCE OF DISEASES*

PROGRAMA GENERAL

10.00 CONFERENCIAS ORALES: SESIÓN 1. TOPIC: Elemental mass spectrometry applications (ICP-MS)
Moderadores: Angel Ríos y Rosa Alonso

10.00-10.10
O1. ***Antonio Bazo****, Eduardo Bolea-Fernandez, Ana Rua-Ibarza, Maite Aramendía, Kharmen Billimoria, Paula Menero-Valdés, Jack Morley, Sara Neves, Armando Sánchez-Cachero, Heidi Goenaga-Infante, Martín Resano. IMPROVING THE QUANTITATIVE ANALYSIS OF SINGLE CANCER CELLS VIA LASER ABLATION ICP-MASS SPECTROMETRY: INTRODUCING A PARTICLE MASS CALIBRATION APPROACH*

10.10-10.20
O2. ***Saida Sanchez-Espirilla****, Belén Callejón-Leblic, Rafael Santana, Antonio Pereira-Vega, Germán Peces-Barba, Tamara García Barrera. ANALYSIS OF SELENOPROTEINS IN LUNG CANCER AND CHRONIC OBSTRUCTIVE PULMONARY DISEASE HUMAN SERUM SAMPLES BY ISOTOPIC DILUTION ANALYSIS COMBINING AFFINITY CHROMATOGRAPHY AND SIZE EXCLUSION CHROMATOGRAPHY COUPLES TO INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY*

10.20-10.30
O3. ***Juan José López-Mayán****, María Carmen Barciela-Alonso, Elena Peña-Vázquez, Antonio Moreda-Piñeiro, and Pilar Bermejo-Barrera. DEVELOPMENT OF ANALYTICAL METHODOLOGIES FOR Ag AND TiO_2 NANOPARTICLES DETERMINATION IN COMPLEX MATRICES*

10.30-12.00 Café + Sesión de Pósters

12.00 CONFERENCIAS ORALES: SESIÓN 2. TOPIC: Chromatography and mass spectrometry developments and applications.
Moderadores: María Luisa Marina y Asier Vallejo

12.00-12.10
O4. **Esther Gómez-Mejía***, María Concepción García, María Castro-Puyana, María Luisa Marina. RECOVERY OF PROTEINS FROM LEMON PEELS BY PRESSURIZED LIQUID EXTRACTION AS A SOURCE OF BIOACTIVE PEPTIDES. CHARACTERIZATION OF THE MOST ACTIVE HYDROLYSATES BY UHPLC-MS/MS*

12.10-12.20
O5. ***Andrea López-Gutiérrez****, N. Rodríguez-Fariñas, R.C. Rodríguez Martín-Doimeadios, Á. Ríos Castro. PROMISING STUDY OF METALLIC NPs IN BIOLOGICAL MEDIA BASED ON EAF4 WITH A MULTI-DETECTOR ARRAY PLATFORM*

12.20-12.30
O6. ***Gemma Beiro-Valenzuela****, L. Olmo-García, S. Rodríguez-Rodríguez, A. Mohamed-Barara, R.P. Monasterio, I. Serrano-García, E. Hurtado-Fernández, R. Pedreschi, A. Carrasco-Pancorbo. DETERMINATION OF ACETOGENINS IN AVOCADO TISSUES FROM BACON, FUERTE, AND HASS VARIETIES BY USING LC-IMS-MS*

12.30-12.40
O7. ***Ana Jano****, Estela Prieto, Adrián Fuente-Ballesteros, Silvia Valverde, Ana María Ares, José Bernal. LIPID PROFILING IN HONEY AND BEE POLLEN USING MALDI-TOF MS*

12.40-12.50
O8. ***Cristina de Dios Pérez****, Ana Ballesteros Gómez, Soledad Rubio Bravo. ANALYZING EMERGING CONTAMINANTS IN INDOOR DUST BY SUPRAS-LC-HRMS*

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13.30-14.30 COMIDA

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14.30-14.40 *O13.* ***Andrea Cabrero-Martín****, Sara Santiago, Verónica Serafín, Ana Montero-Calle, José M. Pingarrón, Rodrigo Barderas, Susana Campuzano. RECOGNITION AND SIGNALING BIOREAGENTS DECORATED GOLD-SILICA NANOCONJUGATES FOR IMPROVED ELECTROCHEMICAL IMMUNOTRACKING OF CXCL12 IN CANCER SCENARIOS*
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15.30-15.40 *O19.* ***Sandra Rodríguez-Blázquez****, Esther Gómez-Mejía, Noelia Rosales-Conrado, María Eugenia León-González. A GREENER FUTURE IN SEED OIL EXTRACTION: SMARTER AND SUSTAINABLE ANALYTICAL STRATEGIES*
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Premio Tesis 2024*:*
OI2. THE ROLE OF ION MOBILITY-HIGH RESOLUTION MASS SPECTROMETRY FOR THE ANALYSIS OF ORGANIC MICROPOLLUTANTS IN COMPLEX MATRICES*.* ***Alberto Celma***

Premio Investigación en 3 min 2024*:*
OI3. INSTALLING CAMERAS IN THE FACTORY: ELECTROCHEMICAL BIOSENSORS FOR EARLY MOLECULAR SURVEILLANCE OF DISEASES. ***Víctor Pérez****, R. M. Torrente-Rodríguez , M. Pedrero, S. Campuzano*

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O4. RECOVERY OF PROTEINS FROM LEMON PEELS BY PRESSURIZED LIQUID EXTRACTION AS A SOURCE OF BIOACTIVE PEPTIDES. CHARACTERIZATION OF THE MOST ACTIVE HYDROLYSATES BY UHPLC-MS/MS. **Esther Gómez-Mejía**, María Concepción García, María Castro-Puyana, María Luisa Marina

O5. PROMISING STUDY OF METALLIC NPs IN BIOLOGICAL MEDIA BASED ON EAF4 WITH A MULTI-DETECTOR ARRAY PLATFORM. **A. López-Gutiérrez**, N. Rodríguez-Fariñas, R.C. Rodríguez Martín-Doimeadios, Á. Ríos Castro

O6. DETERMINATION OF ACETOGENINS IN AVOCADO TISSUES FROM BACON, FUERTE, AND HASS VARIETIES BY USING LC-IMS-MS **M.G Beiro-Valenzuela**, L. Olmo-García, S. Rodríguez-Rodríguez, A. Mohamed-Barara, R.P. Monasterio, I. Serrano-García, E. Hurtado-Fernández, R. Pedreschi, A. Carrasco-Pancorbo

O7. LIPID PROFILING IN HONEY AND BEE POLLEN USING MALDI-TOF MS. **Ana Jano**, Estela Prieto, Adrián Fuente-Ballesteros, Silvia Valverde, Ana María Ares, José Bernal

O8. ANALYZING EMERGING CONTAMINANTS IN INDOOR DUST BY SUPRAS-LC-HRMS. **Cristina de Dios Pérez**, Ana Ballesteros Gómez, Soledad Rubio Bravo

O9. DETERMINATION OF VOLATILE ORGANIC COMPOUNDS IN PEARS BY HS-GC-IMS AND CHEMOMETRIC TOOLS FOR CULTIVAR AND RIPENING STAGE CLASSIFICATION. **Claudia Giménez-Campillo**, Marta Pastor-Belda, Natalia Campillo, Natalia Arroyo-Manzanares, Pilar Viñas

O10. CHILDHOOD OBESITY AND MICROBIOTA. DETERMINATION OF SHORT CHAIN FATTY ACIDS (SCFAS) IN RAT AND MICE FECES BY GAS CHROMATOGRAPHY/MASS SPECTROMETRY. **Arrate Rivas**, Saioa Escondrillas, Omaira de la Hera, Rosa M. Alonso

O11. GUIDED METABOLOMICS FOR THE OPEN DETECTION OF CARBONYL-CONTAINING COMPOUNDS AND ITS APPLICATION FOR DETECTINGPOTENTIAL BIOMARKERS FOR GASTRIC CANCER. **David Fabregat-Safont**, Gabriel Gil-Gómez, Àlex Gómez-Gómez, Élida Alechaga, Laura Visa, Manuel Pera, Óscar J. Pozo

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O15. CHEMILUMINISCENCE MEASUREMENTS USING SMARTPHONES AND DIGITAL IMAGES: DETERMINATION OF HYPOXANTHINE/XANTHINE. **Sonia Alcubierre**, Isabel Sanz-Vicente, Susana de Marcosa, Javier Camacho-Aguayo and Javier Galbán

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Conferencias Orales Invitadas

OI1. BIOELECTROANALYTICAL PLATFORMS WITH MULTIPLEXING AND MULTI-OMICS POTENTIAL FOR PRECISION MEDICINE AT POINT OF CARE

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Advances in medicine, development and accessibility to improved healthcare systems, and social awareness of health, lifestyle and nutrition have led to an unquestionable increase in the population life expectancy in recent decades. However, despite the great achievement it represents, this greater longevity is inevitably leading to an increase in the incidence and prevalence of chronic diseases linked to aging, such as cancer, Alzheimer's disease and diabetes, which require continuous and specialized medical care and are putting increasing pressure on healthcare systems, generating major challenges to their sustainability and response capacity. Furthermore, despite constant advances in the diagnosis and understanding of these diseases, one of the most critical problems is their late detection, usually at an advanced stage. This significantly limits, increases the cost, and restricts the therapeutic options, reducing their efficacy and leading to discouraging prognoses. For this reason, a paradigm shift is underway in medicine, known as precision medicine, which seeks to move from traditional, generalized diagnosis and treatment to a modern approach that can provide the most effective treatment for each patient at the right time.

Aware of the situation and the urgent need to develop new enabling technologies both for the discovery of new biotargets and for their determination in complex scenarios, this PhD Thesis [1] has focused on the design and development of breakthrough electrochemical biosensing tools for the detection of: i) epigenetic biomarkers (DNA/RNA methylations and miRNAs) related to carcinogenic processes; ii) protein biomarkers (tau and TDP-43) involved in the development of neurodegenerative diseases; and iii) biomarkers (insulin and cortisol) for diagnosis and management of diabetes.

All the electrochemical platforms developed have demonstrated their great versatility in performing single or multiplexed analysis of selected biotargets using simple protocols and minimal amounts of reagents and have shown their potential to successfully address practical applications of enormous clinical relevance. For all these reasons, they are considered particularly attractive biotools both to identify specific and unique molecular signatures of each disease and to support their reliable determination, which can contribute decisively to personalized medicine of the three studied diseases of high prevalence, mortality and impact on our society. During this communication I will present the main contributions made during my PhD Thesis and some of the most novel aspects of our current research, continuation of the leading work carried out during my Doctoral Thesis, focused on the development of multiplexed electroanalytical biosensing tools for the evaluation of miRNAs methylome [2] and the four methylation marks involved in the DNA methylation−demethylation cycle [3].

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OI2. THE ROLE OF ION MOBILITY-HIGH RESOLUTION MASS SPECTROMETRY FOR THE ANALYSIS OF ORGANIC MICROPOLLUTANTS IN COMPLEX MATRICES

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Ion mobility separation (IMS) coupled with high-resolution mass spectrometry (HRMS) is emerging as a powerful approach for the non-targeted and suspect analysis of organic micropollutants (OMPs) in complex environmental samples [1]. IMS works by separating ionized compounds based on their mobility through an inert gas under an electric field. The mobility of ions primarily depends on their charge, shape, and size, which allows for the filtration of interfering species such as isomeric or isobaric compounds. As a result, incorporating IMS into analysis improves spectral quality compared to conventional HRMS, especially in complex matrices. Additionally, IMS offers an extra layer of identification through the measurement of the collision cross-section (CCS) values for molecules. These CCS values are matrix- and chromatographic separation-independent, making them a reliable parameter for OMP identification. The development of freely accessible online databases of CCS values for organic molecules is critical to establishing IMS-HRMS as a routine method for environmental screening. This presentation will emphasize the advantages of IMS-HRMS in OMP analysis within complex environmental samples, using real-world examples. Key topics will include the establishment of criteria for reporting identification confidence [2], resolving isomeric and isobaric compounds, and reducing false-positive findings [3]. Additionally, we will explore the spectral enhancement provided by IMS [4] and assess the comparability of CCS values across different instrumental configurations [5].

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OI.3 INSTALLING CAMERAS IN THE FACTORY: ELECTROCHEMICAL BIOSENSORS FOR EARLY MOLECULAR SURVEILLANCE OF DISEASES

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Keywords: *biosensing, electrochemistry, personalized medicine*

Let us imagine the human body as a vast, complex, and continuously operating factory. Each biomolecule performs its role as part of a finely tuned assembly line, contributing to the maintenance of physiological balance. However, when one of these components fails, as is the case with diseases, the machinery does not come to a sudden halt. The malfunction begins silently, and the first indications that something is wrong often do not appear within the factory itself, but rather on the outside: these are the symptoms, which tend to manifest only once significant damage has already occurred.

In this invited talk, awarded as part of the science communication prize granted by the GCTbA-RSEQ in the II Edition of the “Your Research in 3 Minutes” contest, we invite the audience on a journey through the inner workings of this factory, where our allies are high-precision surveillance systems: biosensors. These devices function as molecular CCTV systems, capable of detecting early warning signs well before clinical symptoms emerge.

One of the key steps in our research lies in avoiding constant monitoring of the same section of the factory. Just as a well-planned surveillance system requires cameras at multiple levels to cover various stages of production, our objective is to achieve a comprehensive molecular overview of the body’s internal processes. To this end, we design biosensors capable of detecting a broad range of biomolecular targets including proteins[1,2,3], peptides, DNA, RNA, and their respective modifications[4]. By capturing signals from different layers of biological regulation, we aim to improve diagnostic resolution and provide a complete picture of disease progression, thus enhancing early detection and the personalization of therapeutic strategies.

Developed using biotools, that ingeniously combine magnetic beads, printed electrodes and innovative bioassay and bioreceptor formats, our electrochemical biosensors are specifically designed to identify early indicators of diseases within complex biological matrices. Our research focuses on designing bioplatforms that can operate outside traditional hospital settings, offering rapid, cost-effective solutions that support more preventive, personalized, and accessible healthcare.

Our factory cannot stop. But with the right cameras in place, we can at least detect errors before they spread throughout the entire production line, enabling timely and targeted intervention.

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Conferencias Orales

O1. IMPROVING THE QUANTITATIVE ANALYSIS OF SINGLE CANCER CELLS *VIA* LASER ABLATION ICP-MASS SPECTROMETRY: INTRODUCING A PARTICLE MASS CALIBRATION APPROACH

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Keywords: *single-cell, LA-ICP-MS, gelatin-based calibration, nanoparticles, HeLa cancer cells.*

Cancer is the leading cause of death worldwide, accounting for nearly 10 million deaths per year.[1] As a result, the development of targeted therapies that are less toxic and also entail fewer side effects has become a major focus in cancer research. Recent advances in nanotechnology have led to the development of more effective drug delivery vectors, highlighting those involving noble metal nanoparticles as they exhibit high biocompatibility, tunable size and shape, and modifiable surfaces for drug and ligand attachment. Among them, AuNPs are of especial interest as they have shown great potential as drug carriers, imaging agents, radiosensitizers, and in thermal therapy.[2] In turn, the need for reliable analytical methods to quantify their uptake in single tumor cells has been proved in contrast to conventional bulk analysis, as the latter ones only provide information on the average content within the cell population. Particularly, laser ablation ICP-mass spectrometry (LA-ICP-MS) has developed as a powerful tool for elemental quantitative analysis of individual cells, as it assures that the content of each cell is analyzed individually, overcoming the drawbacks associated to the transport of the cells in suspension that other SC approaches suffer from.

However, LA-SC-ICP-MS is still limited by the difficulties associated with calibration using solid standards. To circumvent this issue, different calibration strategies based on the use of gelatin standards have been proposed in literature. Such approaches rely on preparing gelatin films of different concentrations in either ionic or NP standards so that, as long as the density and thickness of the films are well-characterized, it is possible to calculate the amount of analyte ejected per ablation spot when the entire section of the film is ablated.[3] Self-evidently, this assumptions and prerequisites limit the accuracy and precision of the results, so this work proposes a novel particle mass calibration strategy that is independent of all of them. The fundamental principle of this approach relies on the individual ablation of nanoparticles (NPs) of well-characterized size that are embedded in the films, so that their mass can be directly used for calibration without the need to calculate their exact particle number concentration within the gelatin. To evaluate the performance of the newly developed method in comparison to the other alternatives (ionic and particle number calibration), the three approaches were used for the quantitative analysis of HeLa cancer cells exposed to AuNPs and the results were compared to those obtained by in-suspension single-cell (SC) ICP-MS.

Acknowledgements

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O2. ANALYSIS OF SELENOPROTEINS IN LUNG CANCER AND CHRONIC OBSTRUCTIVE PULMONARY DISEASE HUMAN SERUM SAMPLES BY ISOTOPIC DILUTION ANALYSIS COMBINING AFFINITY CHROMATOGRAPHY AND SIZE EXCLUSION CHROMATOGRAPHY COUPLES TO INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY

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Keywords: *selenoproteins, lung cancer, chronic obstructive pulmonary disease, ICP-MS*

Selenium (Se) is a non-metal with metalloid properties that are of key importance for human health. Among the chemical forms in which Se can be metabolized in the human body, selenoproteins are considered the most important due to their biological roles [1] being biomarkers of Se status [2].

In this study, we determined the absolute concentration of Se in the form of selenoproteins using an analytical method based on two-dimensional separation, affinity (AF) and size exclusion chromatography (SEC) couples to inductively coupled plasm mass spectrometry (ICP-MS) and using isotopic dilution analysis (IDA). Concretely, we determined the Se levels of extracellular glutathione peroxidase (eGPx), selenoprotein P (SELENOP), selenoalbumin (SeAlb), and selenometabolites of 154 serum samples from patients with lung cancer (LC) and chronic obstructive pulmonary disease (COPD) with varying degrees of severity, including COPD patients who developed LC during a 7-year follow-up (COPD-LC). Samples from healthy people were also analysed as controls (HC).

We found significant differences in some selenoproteins in the different groups studied, such as SELENOP in LC and eGPx in COPD, which could point to critical roles of both selenoproteins in health and disease and might indicate an impaired antioxidant defense system in these tobacco-related diseases. Alterations in the concentrations of selenoproteins were also found in COPD-LC patients with mild severity of airflow obstruction. Furthermore, the association between SeAlb and SELENOP in LC groups also merits further study.

Selenoproteins may have potential utility as biomarkers in tobacco-related diseases which are characterized by a significant increase in oxidative stress. In this regard, AUC values greater than 0.75 have been generally considered useful in clinical diagnosis [3]. In our study, eGPx shows some promise in differentiating patients with COPD and LC from patients with airflow obstruction alone. However, due to the possible limitations in sample size, especially in the COPD-LC group, these results should be validated in a larger population. Our data provides novel insights into the role of the selenoproteome in COPD and LC, which may have implications for diagnosis, therapy, and targeted supplementation [4].

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O3. DEVELOPMENT OF ANALYTICAL METHODOLOGIES FOR Ag AND TiO_2 NANOPARTICLES DETERMINATION IN COMPLEX MATRICES

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Keywords: nanoparticles, extraction, silver, titanium dioxide

The widespread and growing use of nanomaterials presents significant analytical challenges, particularly regarding their accurate detection and quantification in complex matrices. To address these challenges, this study aims to develop advanced analytical methodologies for the determination and quantification of silver (AgNPs) and titanium dioxide (TiO_2NPs) nanoparticles.

The research focuses on the optimization, application, and validation of enzymatic and alkaline extraction protocols for isolating nanoparticles from complex biological matrices, particularly marine organisms. The extraction methods were tailored for various matrices, including seaweeds (*Palmaria palmata*, *Ulva* sp.), mussels (*Mytilus edulis*), and turbot (*Scophthalmus maximus*), ensuring efficient recovery while preserving the physicochemical integrity of the nanoparticles under mild conditions.

An ultrasound-assisted enzymatic hydrolysis protocol was optimized for the extraction of AgNPs from *Palmaria palmata* previously exposed to PVP-coated AgNPs, using Macerozyme R-10® as the enzymatic agent. Parameters such as sonication type, amplitude, time, mode, hydrolysis time, and enzyme concentration were thoroughly evaluated.

In parallel, an ultrasound-assisted alkaline extraction method for TiO_2NPs was developed using tetramethylammonium hydroxide (TMAH). The method was optimized by assessing TMAH concentration, extraction volume, and sonication time.

For mussel and turbot tissues, a pancreatin-lipase enzymatic mixture was applied to solubilize lipids and enable the effective release of AgNPs and TiO_2NPs from high-fat matrices.

Finally, the extracted nanoparticles were analyzed using single-particle inductively coupled plasma mass spectrometry (SP-ICP-MS) to determine their concentration and size distribution. Additionally, nanoparticle characterization was supported by transmission electron microscopy (TEM), scanning electron microscopy (SEM), scanning transmission electron microscopy (STEM), dynamic light scattering (DLS), and zeta potential measurements, applied to both stock suspensions and biological extracts.

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O4. RECOVERY OF PROTEINS FROM LEMON PEELS BY PRESSURIZED LIQUID EXTRACTION AS A SOURCE OF BIOACTIVE PEPTIDES. CHARACTERIZATION OF THE MOST ACTIVE HYDROLYSATES BY UHPLC-MS/MS

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Keywords: *lemon peel, biomass valorization, sustainable extraction, plant-based proteins, bioactive peptides, bioactive properties.*

Fruits of the *Rutaceae* family, including lemons, oranges, and mandarins, are among the world's major crops, with global production continually increasing. This growth has led to significant waste, as up to 50-60% of the fruit's weight, primarily the peels, is discarded. Consequently, sustainable management strategies for these residues have become essential and are a priority for both the United Nations and the European Commission [1]. The valorization of citrus peel biomass offers a sustainable and promising alternative, proposing the use of citrus peels as a renewable and cost-effective source of valuable compounds with industrial significance, such as polyphenols, organic acids, terpenoids, and proteins [1]. Protein recovery is particularly noteworthy due to the growing consumer preference for plant-derived proteins [2], which are valued not only for their nutritional benefits but also for their ability to release bioactive peptides. Furthermore, these peptides can serve as bioactive ingredients in various industries [3,4]. Be as it may, citrus peels as a protein source remain relatively under-researched, constituting a promising research area for further study.

To address both issues, this study proposes a green extraction method based on pressurized liquid extraction (PLE) to obtain proteins from lemon peel biowaste. A Box-Behnken experimental design and multi-response surface analysis were employed to determine the optimal PLE conditions, considering key factors such as the ethanol-water ratio (0:100-80:20 %, v/v), temperature (100-180°C), and extraction time (3-15 min). Total protein content and the formation of Maillard compounds were selected as experimental responses. Additionally, the sustainability of the PLE method was evaluated using an analytical greenness metric tool. Subsequently, the protein extract was hydrolyzed with alcalase and thermolysin, and its antioxidant, antimicrobial, and antihypertensive potentials were evaluated *in vitro*, revealing promising results. Finally, the UHPLC-Q-TOF-MS/MS profile showed several bioactive compounds, including peptides, polyphenols and organic acids, demonstrating the potential use of lemon peel extracts in the agri-food and nutraceutical industries.

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O5. PROMISING STUDY OF METALLIC NPs IN BIOLOGICAL MEDIA BASED ON EAF4 WITH A MULTI-DETECTOR ARRAY PLATFORM

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Keywords: *EAF4, metallic nanoparticle, biological media, UV-vis, MALS, ICP-MS*

Gold (AuNPs) and platinum (PtNPs) nanoparticles are being studied for biomedical applications due to their extraordinary physico-chemical properties [1]. Nevertheless, its presence in biological media results in different transformations, such as aggregation/agglomeration, dissolution or even adsorption of different macromolecules as proteins. All of this would affect the surface charge of the NPs and the electrical properties derived from it (e.g. zeta potential and electrophoretic mobility), which provide relevant information about their behavior, fate and toxicological profile [2]. Hence, analytical techniques that allow isolation, quantification, and multi-characterization of the NPs in complex matrices (as biological media) are needed, but they are still in an early stage of development.

For this purpose, a promising alternative is electrical asymmetrical flow field-flow fractionation (EAF4). This emerging technique isolates the NPs in complex media and through its coupling to multiple detectors provides relevant and complementary information about their physico-chemical properties. Nevertheless, it remains underutilized to date. Therefore, in this work we develop a strategy based on EAF4 with a multi-detector array platform (ultraviolet- visible absorption (UV-vis), multi-angle light scattering (MALS), and inductively coupled plasma mass spectrometry (ICP-MS)) for the characterization of AuNPs and PtNPs in complex biological matrices. For this purpose, bovine serum albumin (BSA), fetal bovine serum (FBS), and cell culture media (Dulbecco's Modified Eagle Medium, DMEM) have been used. Results showed an increase in the NP size and changes both in the zeta potential and electrophoretic mobility probably because of the adsorption of proteins on their surface. Also, with ICP-MS for AuNPs an oxidation process in FBS and DMEM was observed.

This work demonstrates that EAF4-UV-vis-MALS-ICP-MS platform can provide crucial information for studying NPs in complex biological media. This characterization technique aids in understanding the NP behavior, which is essential for future biomedical applications.

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O6. DETERMINATION OF ACETOGENINS IN AVOCADO TISSUES FROM BACON, FUERTE, AND HASS VARIETIES BY USING LC-IMS-MS

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Keywords: *Avocado, Acetogenins, LC-IMS-MS*

Acetogenins are bioactive compounds derived from C-18 fatty acids, naturally present in various tissues of avocado (*Persea americana* Mill.) such as peels, pulp, seeds, and leaves. They serve a crucial defensive role against herbivores and fungal infections. Notably, their antimicrobial properties include the inhibition of bacterial endospore germination and potent listericidal activity. These qualities have attracted the food industry's interest, positioning acetogenins as natural alternatives to synthetic additives, aligning with clean labels and sustainable solutions.

The objectives of this study were to optimize the extraction process, determine the optimal conditions for acetogenin separation and detection, investigate their distribution across different avocado fruit tissues, and evaluate their potential role in the fruit ripening process. To achieve these goals, the pulp, peel, and seeds of three avocado varieties —*Bacon*, *Fuerte*, and *Hass*— were analyzed at different ripening stages using a powerful LC-IMS-MS method. A total of 180 samples were examined, with 5 replicates per tissue, variety, and ripening stage, with each replicate consisting of 6 avocado fruits.

The extraction procedure was established after testing different solvents (isopropanol, methanol, and methanol:ether (50:50, v/v)), one or two extraction cycles, and evaluating the need for an additional clean-up step. Based on the results, the selected optimal protocol entailed a solid-liquid extraction with methanol in a single cycle and no clean-up step. The effects of important chromatographic parameters such as column type, flow rate, and temperature were also evaluated. Once the best conditions for the separation and detection of the target analytes were determined, the analytical performance of the method was evaluated and then, it was used for sample analysis.

The comprehensive characterization of the full acetogenin profile in the different evaluated matrices was tackled in a first stage, tentatively identifying 21 relevant metabolites belonging to this chemical family (including several isomers), and 3 fatty acid derivatives that appeared as peaks of certain abundance in the profiles. Subsequently, the quantitative data were analyzed to identify the most abundant acetogenin in each tissue and variety. AcO-avocadene was the predominant acetogenin in *Bacon* seeds, whereas persenone B was most abundant in *Fuerte* and *Hass* seeds. In the peel, persenone A was dominant in *Bacon*, AcO-avocadene in *Fuerte*, and persin in *Hass*. In the pulp, AcO-avocadene was the most abundant compound in *Bacon* and *Fuerte*, whereas persin was predominant in *Hass*. Additionally, the data were further explored using chemometric tools, including Principal Component Analysis (PCA). When each tissue was analyzed separately, the samples naturally clustered by variety, suggesting that acetogenins may serve as potential varietal markers.

This research enhances the understanding of acetogenin's role in avocado by improving extraction and determination methods and conducting a comprehensive analysis across various tissues and cultivar types. In addition, the ion mobility data has facilitated the development of an experimental CCS library, improving the reliability of future metabolite annotation. Moreover, this in-depth study is highly beneficial to the avocado industry as it may aid in the value addition of by-products by investigating the chemical potential of commonly discarded tissues.

O7. LIPID PROFILING IN HONEY AND BEE POLLEN USING MALDI-TOF MS

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Keywords: *MALDI-TOF, honey, bee pollen, lipids, lipid profile.*

Lipids play a fundamental role in biological systems, as they include a wide range of molecules —e.g., triglycerides, sterols, or phospholipids—each contributing uniquely to cellular structure and function. Studying the lipid composition of apicultural products like honey and bee pollen can serve as a useful tool for determining their authenticity and origin, both botanical and geographical. Although matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has proven effective in lipid analysis of various biological and food sources, its potential for characterizing lipids in bee pollen and honey has not yet been fully explored.

In this study, we developed and optimized a methodology for lipid profiling in bee pollen, light honey, and dark honey using MALDI-TOF MS. Different extraction strategies were evaluated, testing a range of solvents (both individual and in mixtures), sample quantities, and extraction techniques including solid-liquid and liquid-liquid approaches. Parameters including extraction time and the use of mechanical aids such as vortex and vibromatic mixing, and ultrasound devices were also assessed. For the MALDI-TOF MS analysis, various matrices, sample-to-matrix ratios, and ionizing agents (Na^+, Ag^+, and I^-) were examined to improve lipid ionization efficiency. The results provide a basis for a reliable and reproducible lipid analysis workflow in both aforementioned beehive products.

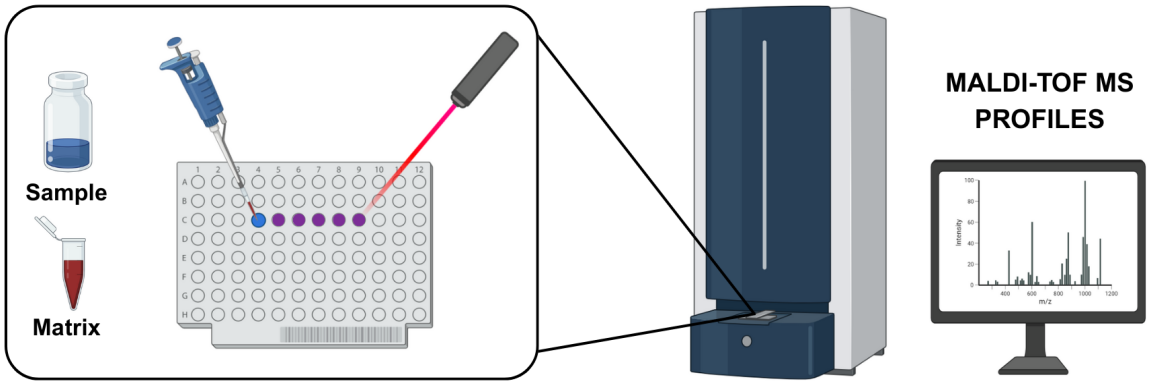

Figure 1. General scheme of MALDI-TOF MS procedure.

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O8. ANALYZING EMERGING CONTAMINANTS IN INDOOR DUST BY SUPRAS-LC-HRMS

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Keywords: *Supramolecular solvent, Drugs, Indoor dust*

Indoor environments, where people spend most of their time [1], are recognized as important reservoirs for contaminants, with settled dust acting as a significant exposure pathway, particularly for vulnerable groups such as children [2]. While pollutants like PCBs, PAHs, and flame retardants have been extensively studied, quantitative data on the presence of drugs of abuse and pharmaceuticals in indoor dust remain scarce.

In this study, we developed a simple, rapid, and sustainable method based on supramolecular solvents (SUPRASs) for the extraction and quantification of these emerging contaminants. SUPRASs, formed by reverse aggregates of 1-hexanol in THF:water mixtures [3], offer versatile extraction abilities due to their nanostructured organization and polarity gradients. Compared with conventional solvents, the use of SUPRAS significantly reduces solvent consumption (~0.2 mL per sample), simplifies sample preparation, and eliminates the need for cleanup steps.

SUPRAS extracts were analyzed using liquid chromatography coupled with high-resolution mass spectrometry (LC-HRMS). A total of 23 target substances—including drugs of abuse, pharmaceuticals, and stimulants were quantified in 43 dust samples collected from various public spaces, such as bars, restaurants, educational institutions, and social centers. Each sample was tested positive for at least one compound. Caffeine and nicotine were detected in all samples at median concentrations of 5720 and 19,380 $ng \cdot g^{-1}$, respectively. Among the drugs and pharmaceuticals, cocaine and paracetamol exhibited the highest detection frequencies (97.8% and 100%), with median concentrations of 232 and 436 $ng \cdot g^{-1}$, respectively. Notably, dust from bars and restaurants had the highest median levels of total drug residues (2491 $ng \cdot g^{-1}$), substantially exceeding those found in pharmacies and educational buildings.

Exposure estimates based on a dust ingestion rate of 30 mg/day indicated median intake values of 0.02–0.4 $ng \cdot kg\ bw^{-1} \cdot day^{-1}$ for drugs, 0.09–0.34 $ng \cdot kg\ bw^{-1} \cdot day^{-1}$ for pharmaceuticals, and 0.99–68.3 $ng \cdot kg\ bw^{-1} \cdot day^{-1}$ for caffeine and nicotine. These values were generally below the thresholds associated with toxicological concern. However, the findings emphasize the need to consider indoor dust as an additional source of unintentional exposure, alongside inhalation and dermal absorption, in future risk assessments.

Acknowledgements

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O9. DETERMINATION OF VOLATILE ORGANIC COMPOUNDS IN PEARS BY HS-GC-IMS AND CHEMOMETRIC TOOLS FOR CULTIVAR AND RIPENING STAGE CLASSIFICATION

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Keywords: *pears, ripening, volatile organic compounds profile, headspace, gas chromatography, ion mobility spectrometry.*

The pear is a globally important fruit, grown in many regions to meet year-round demand [1]. To ensure a continuous supply, strategies such as controlled atmosphere storage, pre-harvest treatments, and staggered cropping are commonly used [2]. Pears are rich in volatile organic compounds (VOCs), which determine their aroma, flavour and texture. These VOCs vary with cultivar and ripening stage and play a key role in the sensory quality and market value of the fruit [3].

The aim of this study was to develop, optimise and validate an analytical method for the determination of VOCs in pears using headspace gas chromatography coupled to ion mobility spectrometry (HS-GC-IMS). The resulting VOC profile was also used to discriminate between different pear cultivars and to assess the optimal ripening stage of Ercolina pears.

A total of 254 samples of *Pyrus communis L.* (European pear) representing the cultivars Blanquilla, Conference, Ercoline and Rocha were analysed. Markers obtained from the integrated topographic maps of each sample were employed in a Partial Least Square Discriminant Analysis (PLS-DA) model to discriminate between cultivars based on their VOC profiles. The model showed excellent performance, with a Q2 value of 0.878 and 100 % classification accuracy in both training and validation sets. Additionally, the analysis allowed the identification of VOC characteristics of each cultivar.

On the other hand, Ercoline pears from Jumilla (Region of Murcia, Spain), recently granted with the Protected Designation of Origin (PDO), were analysed using the optimised HS-GC-IMS method. A total of 227 samples of Ercoline pears, harvested at different ripening stages— from early development to commercial maturity— were studied to assess the changes in the VOC profile during ripening process of the fruit. For the first time, Principal Component Analysis (PCA) and PLS-DA applied to HS-GC-IMS data were used to predict ripening stages of pears, achieving excellent performance with 100 % accuracy in training and validation rates and Q2 of 0.856. Finally, key VOCs associated with both unripe and ripe stages were identified, and the results were supported by heatmap analysis.

This study offers practical value to growers by providing a reliable tool for authenticating pear cultivars, thereby reducing the risk of fraud. It also supports the determination of the optimal harvesting time, helping to ensure fruit quality and prevent economic losses.

Acknowledgements

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O10. CHILDHOOD OBESITY AND MICROBIOTA. DETERMINATION OF SHORT CHAIN FATTY ACIDS (SCFAS) IN RAT AND MICE FECES BY GAS CHROMATOGRAPHY/MASS SPECTROMETRY

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Keywords: *Childhood obesity, short-chain fatty acids (SCFAs), feces, gas chromatography (GC), mass spectrometry (MS).*

Childhood obesity represents one of the serious health problems facing our society today. This disease affects the composition and variety of the intestinal microbiota. The microbiota is made up of a large number of microorganisms, supplies vital nutrients and contributes to the fermentation of carbohydrates to form short-chain fatty acids (SCFAs) that play a crucial role in regulating intestinal balance and have a significant impact on the immune system.[1,2]

This work is part of a project to create SCFA-producing microencapsulates to reduce the risk of childhood obesity. In this context, the objective of this work is the development of an analytical method based on gas chromatography/mass spectrometry (GC/MS) after solid-liquid extraction with methyl tert butyl ether (MTBE) for the determination of SCFAs in feces of mice and rats. The GC/MS method has been validated following the validation guidelines of the European Medicines Agency (EMA).[3]

The concentrations of SCFAs found in the three matrices are at mg/g levels. Acetic acid, propionic acid and butyric acid are the most abundant ones and those with recognized benefits for human health. The obtained results will be applied to the study of the ingestion of microencapsulated symbiotics (prebiotic + probiotic) effect on the intestinal microbiota.

Acknowledgements

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O11. GUIDED METABOLOMICS FOR THE OPEN DETECTION OF CARBONYL-CONTAINING COMPOUNDS AND ITS APPLICATION FOR DETECTING POTENTIAL BIOMARKERS FOR GASTRIC CANCER

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Keywords: *Metabolomics; Gastric cancer; Biomarker; Early diagnosis; LC-MS/MS; Bioanalysis.*

Gastric cancer (GC) is a leading contributor to global cancer mortality, being the fourth leading cause of death by cancer in developed countries with one million new GC patients diagnosed annually[1]. It is often diagnosed at advanced stages and there are no effective therapies for metastatic disease or tumor relapse after surgery with curative intent. So, the availability of GC biomarkers is an unmet medical need for the early diagnosis of the disease and optimization of the treatment. The analysis of the metabolic pathways dysregulated in tumors from different origin has revealed that gastric cancer is one of the tumor classes with higher number of metabolic alterations, especially in the glucose and energy metabolism pathways[2]. Therefore, targeting these pathways can open new avenues in the detection of biomarkers for diagnosis/prognosis of GC.

We have developed a new metabolomics strategy, so-called guided-metabolomics, able to openly detect a specific part of the metabolome[3]. The strategy was focused on the detection of carbonyl-containing compounds, highly prominent in the energy metabolism pathways, based on the chemical derivatization of this moiety and the detection of derivatized compounds by precursor ion scan acquisition mode. Briefly, serum samples were derivatized with o-benzyl hydroxylamine, which reacts with ketones, aldehydes and carboxylic acids. After that, extracts were analyzed by liquid chromatography coupled to tandem mass spectrometry (triple quadrupole) using precursor ion scan acquisition, targeting all the compounds that yielded tropylium product ion (*m/z* 91), as it is the main product ion from the derivatization tag. Finally, mzMine 4.0 was used for feature detection, retention time alignment and data normalization, and statistical analysis was performed with MetaboAnalyst 5.0. The chemical structure of the relevant features was tentatively identified based on observed fragmentation,

The strategy was applied for the detection of relevant features in a GC cohort containing 127 GC cases plus 52 healthy individuals. Changes in the levels of several candidate markers were specifically detected in GC patients, and some of them were firstly identified based on their fragmentation pattern, and then confirmed with reference analytical standards. Analysis of historical controls show that the levels of one non-yet elucidated metabolite are upregulated in patients with GC, but not in patients with other tumors from the digestive tract, indicating that, in addition to candidate diagnostic biomarkers, cancer-specific metabolites can be identified using this approach.

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O12. ANALYTICAL CHARACTERIZATION OF HUMAN BREAST MILK EXOSOMES: IMPACT OF PASTEURIZATION ON THEIR BIOACTIVE COMPONENTS

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Keywords: *human milk, exosomes, composition, pasteurization*

Human breast milk (HBM) is a complex biological fluid that provides macronutrients, micronutrients, and a variety of bioactive compounds (including extracellular vesicles, hormones, oligosaccharides, and immunomodulatory components) that are crucial for infant growth and development. Among these, exosomes are a subpopulation of extracellular vesicles (EVs), ranging from 50 to 200 nm in size, enclosed by a lipid bilayer membrane, and carrying proteins, nucleic acids (DNA and RNA), lipids, and essential elements. These vesicles mediate intercellular communication and regulate critical developmental processes in the neonate [1].

When maternal milk is unavailable, donor human milk (DHM) is widely used to feed preterm infants in neonatal intensive care units. However, DHM undergoes pasteurization to ensure microbiological safety, a process that may alter the bioactive composition and functionality of milk-derived exosomes. Understanding how pasteurization impacts their molecular integrity is essential for optimizing the nutritional and therapeutic value of DHM [2].

This study aims to comprehensively characterize the molecular composition of exosomes derived from raw and pasteurized HBM. Exosomes were isolated using size-exclusion chromatography (SEC) columns and physically characterized through a BCA assay for protein content, as well as Transmission Electron Microscopy (TEM), Dynamic Light Scattering (DLS), and Nanoparticle Tracking Analysis (NTA) to evaluate size distribution, morphology, and concentration. Purity was assessed using size-exclusion high-performance liquid chromatography (SEC-HPLC) coupled with ultraviolet-visible (UV-Vis) detection. The protein composition of EVs was analyzed by nanoHPLC-ESI-MS/MS. In addition, essential trace metal cargo in exosomes was determined using inductively coupled plasma mass spectrometry (ICP-MS), and miRNA expression profiles were analyzed by RT-qPCR. These integrated analytical techniques enable a comprehensive assessment of the impact of pasteurization on the integrity and functionality of human milk exosomes.

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O13. RECOGNITION AND SIGNALING BIOREAGENTS DECORATED GOLD-SILICA NANOCONJUGATES FOR IMPROVED ELECTROCHEMICAL IMMUNOTRACKING OF CXCL12 IN CANCER SCENARIOS

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Keywords: *electrochemical technology; CXCL12; Au@SiO_2 NCs; plasma; colorectal cancer.*

Colorectal cancer (CRC) is one of the leading causes of cancer-related mortality worldwide. Its prognosis is highly dependent on early diagnosis; however, current screening tools often lack the sensitivity and specificity required for its detection at early stages. In this context, the identification of reliable biomarkers and the development of enabling electroanalytical biotechnologies for their simple, sensitive and accurate determination are key priorities in precision oncology.

This communication presents the use of gold-silica nanoconjugates (Au@SiO_2NCs) decorated with biotinylated detection antibodies (b-dAb) and horseradish peroxidase (HRP) for the improved electrochemical immunosensing of chemokine ligand-12 (CXCL12), a relevant marker implicated in colorectal cancer (CRC) progression. Implemented on an amperometric sandwich immunoplatform the resulting nanobioconjugates provided significant signal amplification due to their high enzyme loading capacity and improved antibody orientation. The determinant role played by each of the nanomaterials forming the nanohybrid was evaluated. Under optimized conditions, the developed immunoplatform exhibited a linear response ranging from 72 to 1000 pg mL^{-1}, a 4 times higher sensitivity than the conventional labeling-based immunoplatform, a LOD of 22 pg mL^{-1}, and a storage stability of one month.

The platform was confronted with the analysis of CXCL12 in plasma samples from healthy individuals and CRC patients providing statistically significant content between these two groups. These results highlight the value of the CXCL12 content determined by the technology developed for the minimally invasive diagnosis of CRC.

In addition, both the immunoplatform and the Au@SiO_2NCs can be easily modified with other recognition and/or signaling molecules, allowing the developed technology to be easily transferred to the determination of other targets or its exploitation for multiplexing of several of them in a single assay, which would add precision to the diagnosis and improve therapy efficiency in diseases as complex and heterogeneous as cancer.

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O14. APPLICATION OF A SPHERICAL MESOPOROUS SILICA FUNCTIONALIZED WITH OCTADECYLSILANE LIGANDS AS SORBENT IN THE MINIATURIZED MSPEED TECHNIQUE FOR THE SIMULTANEOUS EXTRACTION OF 23 ALKALOIDS IN FLOWER EXTRACT SAMPLES

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Keywords: *tropane alkaloids, pyrrolizidine alkaloids, microextraction, flower extract supplements, food safety, UHPLC-MS/MS*

The analysis of toxic compounds, such as alkaloids, is crucial due to the risks they pose to human health. Within this group, tropane alkaloids (TAs) can cause acute symptoms, including hallucinations and tachycardia, while pyrrolizidine alkaloids (PAs) are associated with chronic conditions, such as liver damage. These substances are naturally produced by certain plant families as a defense mechanism and can enter the food chain through cross-contamination or be present in dietary supplements made from plants that naturally synthesize them, such as *Borago officinalis*, known for its PA content [1]. To address the analysis of these compounds in complex matrices, there is a growing need for analytical methods that are not only effective but also aligned with the principles of Green Analytical Chemistry (GAC). In this context, the goal is to minimize the use of reagents, solvents, sample amounts, and energy, promoting more sustainable procedures. One key strategy to achieve these objectives is the miniaturization of conventional techniques. A clear example is the µSPEed technique, a miniaturized version of traditional solid-phase extraction (SPE), which requires only a few microliters of solvent and sample. The success of these methodologies largely depends on the materials used in the extraction phase. Therefore, the design and synthesis of new materials, applied in microextraction techniques such as µSPEed, represent a promising alternative, offering excellent performance in terms of selectivity, sensitivity, precision, and accuracy, while adhering to the principles of Green Chemistry.

In this study, a spherical ordered mesoporous silica functionalized with octadecylsilane ligands (SM-C18) was successfully synthesized, showing a surface area of 647 m²/g, a pore size of 45 Å, a particle diameter of 4–6 µm, and a functionalization degree of 0.37 mmol/g. This synthesized material was used as an adsorbent in EPREP micro-solid phase extraction (µSPEed) cartridges designed for use with a handheld programmable digital analytical syringe (digiVOL®). A total of 1.5 mg of the material was packed into each cartridge, which were then used for the extraction of 21 PAs and 2 TAs from flower extract samples marketed as dietary supplements. The material demonstrated higher analyte retention capacity compared to commercial material, and the extraction conditions for the 23 compounds were optimized. The optimal process conditions were as follows: 100 µL of methanol (2 cycles) and 100 µL of water (2 cycles) for conditioning, 100 µL of reconstituted sample in water (10 cycles) for a total sample load of 1 mL, and 100 µL of methanol (2 cycles) for elution, followed by analysis via UHPLC-MS/MS. The optimized methodology was successfully validated, yielding good recoveries ranging between 91% and 97%, low quantification limits, and no matrix effect. Furthermore, the material could be reused for at least 75 extractions. Finally, the validated and optimized methodology was applied to 20 real samples. These samples were extracts prepared from edible flowers and marketed as dietary supplements. In two different batches of samples obtained from *Convolvulus arvensis* flowers (FES4a and FES4b), atropine and scopolamine were quantified [2].

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O15. CHEMILUMINISCENCE MEASUREMENTS USING SMARTPHONES AND DIGITAL IMAGES: DETERMINATION OF HYPOXANTHINE/XANTHINE.

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Keywords: *chemiluminiscence, smartphone, hypoxanthine, xanthine*

The research group has developed a device (through the use of 3D printing technology MSLA -Masked Stereolithography Apparatus-), fully functional, compact, small-sized and easy to transport, which allows light-controlled colour measurements with any Smartphone using coordinates RGB. This device has been used to absorption and fluorescence measurements successfully.

In this work this device is applied to chemiluminiscence measumerents.

Optimisation of the design and measurement conditions (ISO, exposition time. RGB coordinates…) was carried out by measuring chemiluminiscence of the reaction of luminol with H_2O_2 in the presence of a catalyst. Under the best conditions ([luminol]=3.7 x10^{-4}M, [Co(II)]= 1.7x10^{-4}M]), the H_2O_2 calibration is carried out (1.0*10^{-5}M-2*10^{-4}M)

This methodology allows the enzymatic determination of analytes in which H_2O_2 is a reaction product, such as hypoxanthine/xanthine with Xanthine Oxidase (XO), involved in ATP degradation (Figure 1).

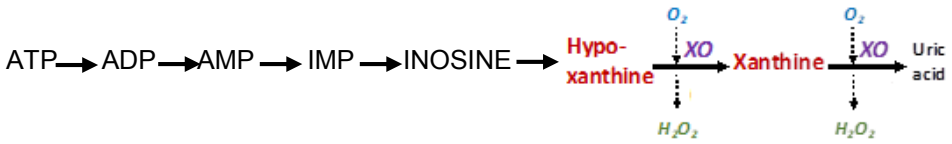

Figure 1. Sequence of enzymatic reactions

Uric acid turned out to be an inhibitor of chemiluminescence, so it was essential to remove it from the medium. Once all the parameters involved in the reaction (concentration and order of addition of reagents, pH, ionic strength, reaction time, etc.) had been studied, this method is able to determine concentrations in the range of 7.5x10^{-6} to 2.5x10^{-5}M of hypoxanthine. The methodology has also been applied in real samples of codfish with good results.

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O16. PROVING THE USE OF MOLECULARLY IMPRINTED NANOPARTICLES IN PROTEIN IMMUNOPRECIPITATION

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Keywords: *Molecularly imprinted nanoparticles, immunoprecipitation, CB_1 receptor.*

This work explores the production and potential use of molecularly imprinted nanoparticles (MIN) as antibody substitutes for protein immunoprecipitation (IPP)[1], which could find extensive application in approaches where small-scale protein purification is desired. IPP is a widely used method for isolating proteins and other biomolecules from cell or tissue lysates. This work focuses on producing MIN against the cannabinoid CB_1 receptor, selected as target protein; however, these protocols may be easily adapted using other peptide fragments targeting other proteins. MIN present remarkable advantages as artificial antibodies, including improved stability, even under harsh conditions and prolonged use. In this regard, this breakthrough addresses key limitations of protein-based antibodies, paving the way for more robust and reliable materials for bioanalytical applications.

To produce high-affinity MIN, solid-phase imprinting has been combined with the epitope approach, imprinting, in an acrylamide-based polymer matrix, a small peptide fraction (15 amino acids) that matches with the C-terminal sequence of the cannabinoid receptor type 1 (CB_1 receptor), which was selected as target protein. The CB_1 receptor is one of the most abundant G protein-coupled receptors (GPCRs) in the mammalian brain, and it is involved in a wide variety of physiological and pathophysiological processes. SEM analysis of polymers revealed uniform particles with a spherical shape and a mean diameter of 90.8±10.9 nm determined by dynamic light scattering. On the other hand, batch rebinding experiments showed a K_D (equilibrium dissociation constant) and a B_{max} (maximum binding capacity) of (6.78±3.83) $x10^{-8}$ M and 0.22±0.07 μmol g^{-1}, respectively; an affinity value at the nM scale, similar to that of natural antibodies.

Produced MIN were tested in IPP experiments incubating them in the presence of a recombinant GST-CB_1 protein[2], which includes the amino acid sequence 414-472 from the receptor's C-terminus fused to a glutathione S-transferase (GST) tag. Then, the MIN-protein complex was pulled down adding streptavidin-coated magnetic beads to the mixture, which bind to MIN previously biotinylated on their surface. This enabled the separation of the MIN-protein complex from the solution by simply using a magnet, followed by the denaturation of the bound GST-CB_1, SDS-PAGE separation, and Western blot analysis. IPP efficiency was found to be 11.48% relative to the initial protein concentration, confirming the strong capture capability of the produced MIN.

Acknowledgements

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O17. A novel hybrid LFIA-electrochemical platform for ultra-sensitive *E. Coli* O157:H7 detection

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Keywords: *Lateral flow immunoassay, electrochemical, Escherichia coli.*

A rapid and highly sensitive detection method for *E. coli* O157:H7 is crucial due to its significant impact on agriculture, food safety, environmental sciences, and medicine. This pathogenic strain produces Shiga-like toxins, primarily transmitted through contaminated food or water, that can cause severe foodborne illnesses, including hemorrhagic diarrhea and acute kidney failure [1]. Conventional detection methods, including culture-based techniques, polymerase chain reaction (PCR), and ELISA, are widely employed but they are often time-consuming, labor-intensive, and require specialized personnel. This highlights the critical need for faster, cost-effective, and user-friendly detection technologies to enhance public health and safety.

In this context, our research group has developed a hybrid detection method, combining a traditional lateral flow immunoassay (LFIA) for *E. Coli* O157:H7 based on gold nanoparticles (AuNPs) modified with different biopolymers containing an electrochemical probe [2]. This approach enables direct electrochemical detection with screen-printed carbon electrodes (SPCE), converting the semi-quantitative optical signal into a measurable qualitative signal, proportional to the bacterial concentration. The combination of these techniques has resulted in an enhanced detection platform with an improved sensitivity compared to conventional LFIAs. The developed methodology has a linear range of detection from 10 to 10^4 CFU/mL and a limit of detection (LOD) of 8 CFU/mL. Considering that current regulatory standards demand the absence or low levels of Shiga toxin-producing *E. coli* (STEC) in food and water samples [3], our findings represent a significant advancement toward the development of practical biosensing screening tools.

This method not only enables rapid and sensitive detection but also constitutes a robust and accessible tool that could be adapted to detect other bacterial pathogens by modifying the specific antibodies employed, highlighting its potential as versatile solution for microbial surveillance.

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O18. BIOCATALYTIC ZIF-8 SURFACE-FUNCTIONALIZED MICROMOTORS FOR COPPER SENSING IN CEREBROSPINAL FLUID SAMPLES FOR ALZHEIMER DIAGNOSIS.

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Keywords: *Micromotor, biocatalytic, copper, catalase and monitoring.*

Alzheimer's disease (AD) is the major cause of irreversible dementia in the elderly population worldwide and one of the major causes of the decrease in the quality of life. Efficient diagnosis and monitoring would allow a fast treatment to delay the appearance of symptoms[1]. Herein, zeolitic imidazole framework (ZIF-8)@Au@catalase micromotors are described for motion-based sensing of copper as a marker of AD. The synthesis design was based on enzyme covalent immobilization instead of encapsulation to maximize the contact with the sample at the microscale for the potential use of extremely low AD-diagnosed sample volumes. The micromotors are prepared by asymmetric modification of ZIF-8 with a gold layer for functionalization of catalase as a compatible biocatalyst. The micromotors can propel at speeds of up to 287 ± 41 μm s^{-1} in cerebrospinal fluid (CSF) samples of healthy volunteers. Yet, in the presence of copper, catalase poisoning results in a decrease in the speed that can be monitored for motion-based sensing detection, as illustrated in the analysis of CSF samples from AD patients from mild to severe stages (Braak III to Braak VI)[2]. The copper-mediated modulation of catalase activity proposed here as an indicator of progression states in AD disease possesses distinct advantages such as ultrafast analysis (less than 1 min) and requiring only 1 μL of sample, holding considerable promise as a supporting prescreening tool for fast diagnosis of AD and other neurodegenerative diseases.[3]

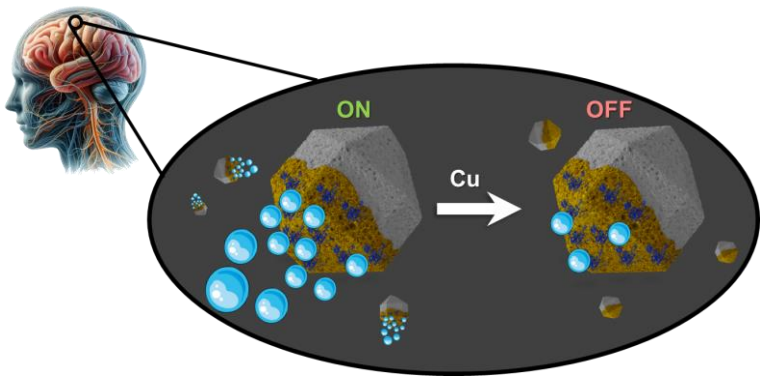

Figure 1. Schematic of ZIF-8 composition and catalytic movement of the ZIF-8@Au@catalase micromotors through CSF.

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O19. A GREENER FUTURE IN SEED OIL EXTRACTION: SMARTER AND SUSTAINABLE ANALYTICAL STRATEGIES

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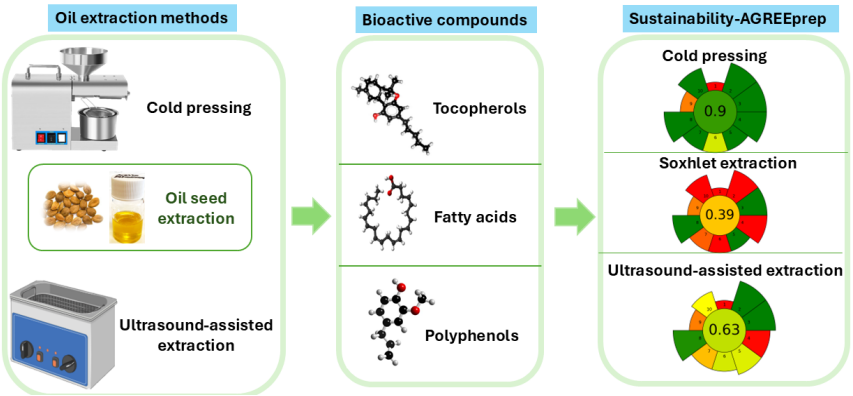
Keywords: *seed oils, bioactive compounds, ultrasound-assisted extraction, cold pressing, AGREEprep software, chromatographic techniques, chemometric tools*

In recent years, agri-food waste, particularly seeds, has gained significant attention due to its potential as a valuable source of bioactive compounds. Seed residues, often discarded during fruit processing, are rich in unsaturated fatty acids, tocopherols, phenolic compounds and other bioactive molecules with promising applications in the cosmetic, pharmaceutical and nutraceutical industry [1]. However, despite their potential, the exploitation of these residues remains limited due to challenges in sustainable extraction methods.

Traditional methods such as Soxhlet extraction, although effective, present drawbacks in terms of energy consumption, the use of flammable and toxic organic solvents, such as *n*-hexane, and waste generation. As a result, the demand for innovative and sustainable methodologies is growing, with a particular focus on cold pressing and ultrasound-assisted extraction (UAE). These techniques aim to enhance the efficiency of bioactive compound extraction while reducing environmental impact [1,2].

Therefore, this work aims to explore alternative extraction methods for seed oil recovery, focusing on cold pressing and UAE using mixtures of green solvents, primarily tert-butanol and ethanol. Experimental design, response surface methodology and multifactorial analysis of variance are employed to determine optimal yield conditions. Additionally, the sustainability of the extraction processes is evaluated using AGREEprep, a tool designed to assess the environmental and economic impacts of each extraction process [3]. The findings demonstrate the feasibility and promising applicability of cold pressing and UAE as greener alternatives to Soxhlet extraction, contributing to the development of greener processes to obtain oils enriched in bioactive compounds useful in the food and cosmetics industries.



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O20. INNOVATIVE DENDRIMER-BASED DNA BIOSENSOR FOR HIV DETECTION

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Keywords: *DNA dendrimer, few-layer bismuthene (FLB), Azure A (AA), DNA biosensor, Human Immunodeficency Virus (HIV)*

The human immunodeficiency virus (HIV) is a lentivirus that directly attacks the human immune system, reducing the body's ability to fight all kinds of diseases. For this reason, prevention, early diagnosis and effective treatment of the infection are essential for health. The most used techniques for detection have certain limitations, so the search for alternative methodologies such as DNA biosensors to overcome these limitations is of great interest within the scientific community. For this reason, the present work proposes the development of a DNA biosensor based on two-dimensional materials and DNA dendrimers for the selective and sensitive detection of the HIV virus. The development of the biosensor is based on the modification of carbon screen-printed electrodes (CSPE) with bismuthene nanolayers (FLB), generating an improved platform for the subsequent immobilization of the synthesized DNA dendrimer that includes the capture probe. Virus detection is carried out by differential pulse voltammetry (DPV), using Azure A (AA) as an electrochemical indicator of the hybridization event. The dendrimer-based DNA biosensor developed presents a limit of detection of 4.81 fM and a linear range from 4.81 fM to 10.0 pM. It was validated with the detection of the virus in human serum samples.

The use of new nanomaterials such as FLB, or DNA structures such as DNA dendrimers, provides biosensors with high stability, sensitivity and selectivity, making them a potential alternative to the methodologies used for the detection of viruses such as HIV.

O21. NEXT-GENERATION BIOANALYTICAL TOOLS FOR THERAPEUTIC DRUG MONITORING OF IMMUNOSUPPRESSANTS IN ORGAN TRANSPLANTATION

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Keywords: *Immunosuppressants, point-of-care, recombinant receptors, (bio)luminescence*

The survival of transplant patients critically depends on the administration of immunosuppressive drugs to prevent organ rejection. These compounds, such as mycophenolic acid (MPA) and tacrolimus (FK506), have a narrow therapeutic window, necessitating rigorous analytical monitoring to maintain blood concentrations within a safe and effective range. Both underdosing and overdosing carry serious clinical consequences, including organ rejection or severe immunosuppression, respectively. Additionally, the pharmacokinetics of immunosuppressants exhibit significant inter- and intra-individual variability, requiring regular and accurate monitoring to optimize therapeutic outcomes and minimize adverse effects.[1] Liquid chromatography–tandem mass spectrometry (LC-MS/MS) is the gold standard for the precise quantification of these drugs in clinical laboratories.[2] However, the development of point-of-care (POC) devices—essential for timely and decentralized testing—typically relies on fluorescence or chemiluminescence-based immunoassays due to their portability and ease of use.

This work explores various analytical strategies for the detection and quantification of MPA and FK506, aiming to bridge the gap between centralized high-precision analysis and accessible, rapid diagnostics at the point of care. For MPA, two complementary strategies were developed: one based on cyclic peptides identified via phage display and genetically fused to a bioluminescent enzyme [3] for use in competitive immunoassays, and another employing a fluorescence polarization assay using a near-infrared dye-labeled MPA analogue[4]. Both approaches enabled ultra-sensitive and highly specific detection of MPA, suitable for rapid and reliable therapeutic monitoring of this drug in blood samples. For FK506, utilizing the high-affinity FKBP1A natural receptor conjugated to a fluorescent protein tag, we have established two highly versatile biosensing platforms for the detection of FK506, which are seamlessly integrated into a bead-based[5] assay and an advanced micromotor[6] format. Furthermore, to aid in the integration of these systems into real-time point-of-care devices, surface functionalization of cyclic olefin copolymer (COC) substrates was carried out with an amino dextran–lipase conjugate (ADLC),[7] creating a strong and adaptable interface for immobilizing drug analogues and improving assay performance.

Acknowledgements
This work was funded by the Spanish Ministry of Science and Innovation through program PID2021-127457OB-C21 and PDC2023145935-C22.

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O22. DEVELOPMENT OF A NOVEL BIOANALYTICAL SYSTEM BASED ON NANOCHANNELS FOR THE LIVE MONITORING OF VIRULENCE MARKERS IN BACTERIAL CULTURES

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Keywords: *Electrochemical detection, virulence factors, real-time monitoring*

Antibiotic resistance in bacteria is becoming an increasingly serious global health issue, posing major challenges in the development of new therapeutic agents [1]. Traditional approaches such as disk diffusion and broth microdilution present limitations due to their time-consuming nature and limited sensitivity to subtle changes [2]. The development of faster and more sensitive strategies for antibiotic testing is imperative.

There has been a growing interest in biosensors as viable alternatives for antibiotic assessment, particularly of electrochemical platforms due to their high sensitivity, rapid detection capabilities, and potential for integration into point-of-care diagnostics. Recent advances enable real-time detailed monitoring of bacterial activity, making them excellent tools for the evaluation of therapeutic agents [3]. Despite progress, *in vivo* electrochemical evaluation remains challenging, since conventional electrochemical probes have limited biocompatibility, which can compromise the bacterial viability.

In this context, we propose a novel electrochemical platform for rapid, real-time *in vivo* monitoring of virulence factors secreted in bacterial cultures. Biocompatible nanoporous alumina membranes functionalized with antibodies against specific secreted virulence factors are used for culturing bacteria. Measurements are carried *in vivo* placing an indium tin oxide (ITO)-coated polyethylene terephthalate (PET) electrode below the membrane, which is also a well-known biocompatible material [4]. In the presence of bacteria, the antibodies capture the secreted antigens, causing a disruption in the electrochemical transport through the nanochannel [5]. The blockage can be monitored in real time using chronoamperometry.

Key experiments were conducted to establish optimal conditions for simultaneous bacterial cultivation and electrochemical measurements. Initially, the red-ox behavior of various red-ox indicator solutions was assessed in the Tryptic Soy Broth (TSB) medium. Additionally, the potential toxicity of these indicators toward the bacteria *Pseudomonas aeruginosa* and *Staphylococcus aureus* was evaluated to ensure their suitability for *in vivo* electrochemical analysis.

Our results pave the way for the real-time in vivo evaluation of therapeutic agents with antivirulence activity, offering a promising tool for assessing treatment efficacy.

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O23. NOVEL AUTOMATED POINT-OF-CARE ANALYSER FOR AMMONIUM ION DETERMINATION IN WHOLE BLOOD

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Keywords: *Ammonium ion, Point-of-care, Whole blood, Potentiometry.*

High levels of NH_4^+ in blood constitute a condition known as hyperammonemia, which is the main pathological trait of some inborn errors of metabolism (IEM). They are characterized by the impairment of the ammonium elimination due to the mutation of some enzymes[1]. Consequently, NH_4^+ increases over the healthy levels, which reach up to 50 µmol L^{-1} in adults and children and up to 100 µmol L^{-1} in newborns. Concentrations higher than 200 µmol L^{-1} constitute severe hyperammonemic states which may lead to poor neurological outcomes and permanent brain damage. Even higher concentrations are related to hyperammonemic comma and death. Besides IEM, there are other non-congenital conditions that may cause hyperammonemia, either due to a decreased detoxification as in cirrhosis and hepatic failure, or due to an increased production of the molecule as in the use of some drugs or in bacterial overgrowth[2]. In order to avoid severe affectations to the neurological system it is imperative to detect and treat hyperammonemic episodes in a reliable and fast manner. Nowadays, sophisticated, expensive and large equipment is used for the analysis of blood NH_4^+ in reference hospitals, based on enzymatic spectrophotometric analytical methods[3]. They require expert personal to obtain plasma, as whole blood is not a suitable sample, and this procedure takes at least 15 minutes. In addition, NH_4^+ quickly increases in blood samples. For all these reasons, more portable equipment that allows the analysis of whole blood is required to permit its implementation at the bedside of the patients for point-of-care (POC) analysis.

This work focuses on the design, optimization, and validation of a fully automated continuous flow analyser, composed by a detection module, a fluid management module and a data acquisition and communication module. The POC system was installed in the laboratory of the Hospital Sant Joan de Déu (HSJD, Esplugues de Llobregat) for its validation under continuous use for a period of two months, analyzing 283 blood samples in parallel with the reference method. Results show a good correlation between the developed potentiometric analytical system and the reference method currently used by the HSJD. Therefore, this POC system constitutes a promising candidate for the determination of healthy and pathological levels of NH_4^+ in blood samples aimed at the monitoring of diseases characterized by hyperammonemia episodes.

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O24. ON-SURFACE ISOTHERMAL AMPLIFICATION FOR ELECTROCHEMICAL DETECTION OF OVEREXPRESSED COLORECTAL CANCER-RELATED LONG NON-CODING RNAs

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Keywords: *lncRNA, liquid biopsy, isothermal amplification, electrochemical detection*

Long non-coding RNAs (lncRNAs) have emerged as promising biomarkers for liquid biopsy. These RNAs, which exceed 200 nucleotides in length and do not encode for proteins, may exhibit variations in their expression levels associated with human pathologies, making them useful for the screening of various diseases, including colorectal cancer.[1] The most widely used technique for investigating the expression patterns of lncRNA is quantitative reverse transcription polymerase chain reaction (RT-qPCR). However, there is a growing need for alternative methods that can decentralize these analyses, especially for point-of-care applications.[2]

Isothermal nucleic acid amplification combined with **electrochemical detection** offers ultralow detectability while simplifying the necessary equipment, as it does not require thermal cycling.[3] This approach enables decentralized analysis by developing genosensors that integrate recombinase polymerase amplification (RPA), an isothermal amplification technique, on gold surfaces with chronoamperometric detection. Two different genosensors have been developed for the relative quantification of a cancer-associated lncRNA with respect to a stable transcript used as a reference.

Methodology optimization was performed with synthetic oligonucleotides, achieving femtomolar detectability after 30 minutes of amplification at 40°C. The developed assays were tested in cell lysates after a selective RNA extraction step involving oligonucleotide-functionalized magnetic particles and subsequent reverse transcription. The results from RPA were compared to those obtained from RT-qPCR.

Acknowledgments

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O25. ELECTROELISA PROBE: A HANDHELD LOW-COST PROBE FOR ELECTROCHEMICAL READOUT OF MICROTITER PLATES

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Keywords: *Electrochemical cell, Decentralized analysis, Infectious mononucleosis, Matrix metalloproteinase 9 (MMP9)*

Enzyme-Linked Immunosorbent Assays (ELISAs) remain indispensable tools in bioanalysis due to their exceptional sensitivity, specificity, and versatility. They are widely employed not only in clinical diagnostics but also in food safety, quality control, and environmental monitoring. Their ability to provide multiplexed measurements when conducted on microtiter plates further enhances their usefulness. However, a significant limitation of conventional ELISAs is their dependence on plate readers, which usually restricts their use to laboratory settings and thus limits its use in point-of-need analysis—an essential factor for enabling rapid decision-making in critical situations.

To address this limitation and facilitate fast and accessible determinations, this work presents the development of a portable electrochemical probe designed for on-site ELISA readout. This probe uses metallic pins as electrodes[1],enabling the fabrication of a cost-effective, user-friendly device that allows rapid and straightforward detection. This innovation has the potential to transform ELISA applications by extending their usability to resource-limited settings and their use in fast diagnosis within healthcare facilities that do not present their own analysis laboratory facilities.

To achieve this, various mass-produced commercially available materials were assessed for their suitability as electrodes, alongside different surface modification strategies. The outcome was an integrated electrochemical probe, specifically designed to fit the wells of standard microtiter ELISA plates. This adaptability ensures seamless compatibility with existing ELISA protocols while offering an alternative electrochemical detection method. Furthermore, this probe can be easily adapted to any microtiter plate assay which can be determined by electrochemical techniques.

The applicability of this innovative electrochemical cell was demonstrated through its use in the potential diagnosis of Epstein-Barr virus (EBV) infection, achieved by detecting human IgG antibodies against EBV. Additionally, it was employed for the quantification of matrix metalloproteinase 9 (MMP9), a biomarker for stroke severity and hemorrhagic transformation. These successful applications highlight the potential of this low-cost, portable, and easy-to-use ELISA reader, not only for clinical diagnostics but also for other scenarios requiring on-site analytical determinations. By eliminating the need for bulky plate readers, this development paves the way for more accessible immunoassay testing across various fields.

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O26. AQUAPHOTOMICS: A BREAKTHROUGH IN ANALYTICAL METHODOLOGIES

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Keywords: *near infrared spectroscopy, water fingerprint*

Aquaphotomics is an innovative technique based on the study of the structure and functions of water and its relationship with vital phenomena. This approach focuses on understanding the absorption patterns of water molecules, analyzing their interactions and behaviors in various biological and chemical systems [1]. By capturing the unique "water fingerprint," aquaphotomics allows for precise and non-invasive characterization of biological samples, including body fluids, food products, and other complex systems.

Within the near infrared region (NIR) region (typically 1300–2500 nm), water exhibits distinct overtone

and combination bands that are highly sensitive to its hydrogen-bonding network and interactions with solutes. Aquaphotomics exploits this sensitivity by systematically analyzing variations in these water absorbance bands, transforming NIR spectroscopy (NIRS) from a conventional compositional analysis method into a functional tool for assessing system state. Rather than treating water as spectral noise or a baseline to be corrected, Aquaphotomics positions water as the main informative component. This approach enhances the analytical resolution of NIRS, allowing for the detection of subtle, system-wide changes through water structure reorganization, even in highly complex biological matrices.

This singular approach could serve as a valuable diagnostic resource, enabling real-time monitoring of biological states by analyzing the spectral signature of water.

Acknowledgements

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O27. ENGINEERING PROTEIN FOLDING FOR REAL TIME, CONTINUOUS BIOSENSING

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Keywords: *Protein engineering, nanobodies, conformational signaling.*

Performing measurements in complex biological media poses significant challenges, such as unspecific biofouling and interference. As a result, many analytical technologies require complex and labor-intensive protocols involving multiple steps, which is impractical for continuous, point-of-care monitoring. As a result, they fail to capture the dynamic nature of physiological homeostasis, hindering our ability to monitor health conditions or adjust drug dosing to metabolic fluctuations.

Currently, only a few successful examples of continuous biosensors exist, with the glucose biosensor being the most well-known. However, this biosensor depends on a specific enzymatic reaction, restricting its application to other targets. Meanwhile, antibodies are easy to produce and develop against any target with high affinity and specificity, yet they lack an intrinsic signal transduction mechanism, limiting their use in continuous biosensing[1].

To address the challenge of enabling continuous molecular detection, we are developing a sensing technology that (i) allows reagent-less molecular measurements; (ii) specific and selective enough to perform directly in biological fluids; and (iii) generalizable to many different targets. Inspired by biophysical natural mechanisms, we use conformational signaling. By coupling analyte binding to a structural change in the receptor, this signaling mechanism allows for straightforward signal transduction. To achieve this, we use nanobodies, single-domain antibodies with a simple and small scaffold that facilitates their engineering into conformational receptors[2].

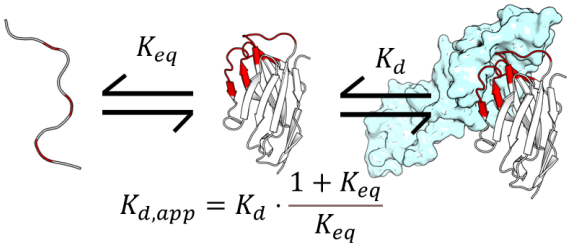

Figure 1. Nanobody-based conformational receptor, in which the binding of the ligand is coupled to a folding conformational change.

As our first proof of concept, we have developed a nanobody-based conformational biosensor for the pregnancy biomarker chorionic gonadotropin (hCG). To do this, we have introduced destabilizing mutations into an anti-hCG nanobody, tuning the folding stability of the nanobody to couple binding to folding. Then, by functionalizing the nanobody with a fluorescence probe, we have developed an optical sensor for measuring hCG concentrations.

Given that nanobodies can be generated for any molecule, and that conformational signaling is a generalizable signaling mechanism, their use will maximize the versatility of our approach for the detection of a wide range of analytes, including disease biomarkers, drugs, hormones and metabolites. Such a tool will improve the development of new biosensors for continuous monitoring, advancing the implementation of personalized medicine.

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Pósters

P1. CAN THE BASAL CHEMICAL PROFILE OF OLIVE CULTIVARS PREDICT THEIR TOLERANCE TO *VERTICILLIUM DAHLIAE*?

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Keywords: *Olea europaea L., LC-MS profiling, TIMS, olive roots, olive stems, olive leaves, pathogen resistance, Verticillium wilt of olive.*

Verticillium wilt of olive (VWO), caused by the soil-borne fungus *Verticillium dahliae*, is among the most damaging diseases affecting olive cultivation worldwide, leading to plant collapse and significant agricultural losses. Since no effective chemical treatments are currently available, identifying naturally resistant olive cultivars has become a central element in integrated disease control strategies.

This research focused on uncovering the metabolic underpinnings of resistance to VWO by employing a comprehensive, non-targeted metabolomics approach. Samples of roots, stems, and leaves were collected from 43 olive phenotypes with varying degrees of resistance, using three healthy, one-year-old plants per cultivar as biological replicates. In total, 387 organ-specific samples were analyzed using a state-of-the-art UHPLC-ESI-TimsTOF MS/MS methodology.

In the first phase of the study, the metabolic profiles of roots, stems, and leaves were comprehensively characterized. More than 80 metabolites were annotated across multiple classes, such as organic acids, iridoids, simple phenols, coumarins, flavonoids, lignans, secoiridoids, triterpenes, and fatty acids. Distinct organ-specific metabolite distributions were noted. Notably, the implementation of ion mobility spectrometry enabled the resolution of isomeric compounds, representing the first determination of such species in olive tissues. In addition, the data contributed to building a preliminary $^{TIMS}CCS_{N2}$ experimental spectral library to enhance future metabolite identification.

In the second phase, multivariate statistical analyses were applied to explore metabolic differences among the cultivars in relation to their resistance to VWO across the three organ types. Clear metabolic distinctions were observed between highly resistant (HR) and susceptible (S) genotypes. Principal component analysis (PCA) revealed natural clustering of samples based on resistance level. Then, supervised statistics (partial least squares-discriminant analysis -PLS-DA-) were used to further discriminate genotype groups.

These classification models pointed out key metabolic markers associated with VWO susceptibility and contributed to defining their compositional patterns. In stems of HR genotypes, secoiridoids and flavonoid derivatives emerged as key discriminant compounds, while lignan-related metabolites contributed to the separation of S genotypes. In leaves, HR cultivars were differentiated by specific profiles involving iridoids and fatty acids, whereas a broader array of compounds -including flavonoids, simple phenols, and fatty acids- played a role in distinguishing S genotypes.

These results demonstrate the analytical power of non-targeted metabolomics combined with ion mobility spectrometry for resolving complex plant metabolomes. The integration of high-resolution separation techniques and multivariate analysis provided a robust framework for biomarker discovery, offering valuable insights for both functional studies and applied breeding programs focused on disease resilience.

P2. SYNTHESIS, CHARACTERIZATION AND POTENTIAL OF SORBITAN MONOPALMITATE-BASED BIOSUPRASs IN THE VALORIZATION OF URBAN PRUNING WASTE

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Keywords: *supramolecular solvents, pruning waste, bioactive compounds*

In recent years, urban pruning waste has gained significant attention as a valuable resource, emphasizing the need for its sustainable and efficient management. Plant biomass serves as an important source of bioactive compounds, such as polyphenols, carotenoids, and anthocyanins. Specifically, pruning waste from white mulberry (*Morus alba*), an ornamental tree commonly used in urban landscaping across Spain, is particularly rich in polyphenols. These compounds exhibit antioxidant, anti-aging, and anticancer properties, making them highly attractive for the cosmetic, pharmaceutical, and food industries[1]. Traditional methods for bioactives extraction from biomass are based on organic solvents. The main drawbacks are the limitation of polarity range for extraction, high solvent consumption, and long extraction times. Moreover, solvents have to be removed from the extracts before being applied to industrial use.

Supramolecular solvents (SUPRAS) are nanostructured liquids formed by the self-assembly of amphiphilic molecules[2]. Their unique properties, such as ability to solubilize compounds across a wide range of polarities, multiple types of interactions, high amphiphile concentration, and tunable properties, make them excellent alternatives to traditional organic solvents for extraction processes[3]. SUPRASs enable *all-in-one* extractions, preserving the original polyphenol profile of the biomass, which is essential since synergistic effects among minor compounds can play a significant role in their healthy properties.

This study presents a novel supramolecular biosolvent (bioSUPRAS) synthesized from a mixture of sorbitan monopalmitate (Span 40), 1-propanol, sodium sulfate, and water. All components are widely used in cosmetic formulations, making the developed bioSUPRAS suitable for industrial applications. The phase diagram of the new bioSUPRAS was investigated to define the environmental conditions necessary for its formation, varying the Span 40 concentration (0.1–0.2% w/v) and the 1-propanol to aqueous sodium sulfate solution ratio (0.14–1.4 M) (2/8–8/2 v/v). The morphology of the resulting nanostructures was analyzed using several microscopy techniques (TEM, cryo-TEM, and cryo-SEM), revealing that the bioSUPRAS nanostructures organized into hexosomes ranging from 0.2 to 2 µm in size. Additionally, the chemical composition of the bioSUPRAS was studied, including its water, sodium sulfate, and 1-propanol content. BioSUPRAS was applied to the extraction of bioactives from white mulberry pruning waste. The procedure was straightforward: 200 mg of dried and milled white mulberry leaves were extracted with 1.5 mL of bioSUPRAS using vortex agitation for 10 min, followed by centrifugation at 15,000 rpm for 10 min to speed up phases separation. The bioSUPRAS extracts were rich in polyphenols, with concentrations up to twice as high as those obtained using traditional organic solvents. Moreover, the bioSUPRAS extracts can be immediately applicable to the cosmetic industry. Overall, the developed extraction method is simple, efficient, cost-effective, and environmentally sustainable.

Acknowledgements

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P3. A TWO-IN-ONE APPROACH: ENHANCING QUANTITATIVE ANALYSIS OF SOLID MATERIALS VIA TANDEM LA/LIBS COUPLED TO ICP-MS

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Keywords: *LIBS, LA-ICP-MS, calibration, internal standardization, polymers*

Over the past decade, advances in ICP-MS technology – particularly in the development of more sophisticated mass analyzers – have been substantial. As a result, the technique's ability to overcome spectral overlap and to perform high-speed single or multi-element monitoring has greatly improved. However, despite these continuous and significant advances in mass spectrometer configurations, the sample introduction system has remained largely unchanged, and the technique continues to focus primarily on the analysis of aqueous solutions.

Alternatively, laser ablation (LA) is typically used as a means of solid sample introduction. However, LA-ICP-MS analysis still faces important challenges that need to be appropriately addressed [1]. Among them, matrix effects strongly influence the reliability of the results, thus requiring the development of suitable strategies to correct matrix-induced bias. Internal standardization is frequently used for this purpose, but certain internal standards, such as carbon, have been reported to exhibit transport and ionization behaviors that differ from those of the analytes, while their use also requires prior knowledge of their content in the samples of interest [2]. Recently, the combination of LIBS and LA-ICP-MS in a tandem configuration has been introduced, allowing for an improvement in the direct quantitative analysis of solid samples [3].

In this work, the potential of a tandem LA/LIBS setup for the quantitative analysis of polymer samples has been investigated. LIBS can potentially provide information for identifying the polymer type, and some of its signals can also offer insights into the ablated material, thus opening new possibilities for internal standardization in LIBS/LA-ICP-MS. Following method development and optimization of the instrumental parameters and operating conditions under compromise settings, the capabilities of this two-in-one approach have been evaluated through the analysis of a series of polymeric certified reference materials differing in type and elemental composition.

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P4. DETERMINATION OF BETAINES AND RELATED COMPOUNDS IN BEE POLLEN BY UHPLC (HILIC)-MS/MS

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Keywords: *bee pollen, betaines, food authenticity, HILIC, method validation, UHPLC-MS/MS.*

Growing consumers´ interest in product origin and authenticity has driven the search for specific markers to detect possible adulterations in bee products, like bee pollen. Among potential markers of bee pollen are betaines, quaternary ammonium compounds present in plant species, which are formed through biosynthetic pathways catalyzed by enzymes. Betaines have attracted the interest of the scientific community due to their relevance in essential metabolic and physiological processes in both animals and plants. Briefly: maintaining the homeostasis of organisms or contributing to the regulation of key functions for cellular stability and adaptation to different environmental conditions among others. Betaines are naturally occurring substances in bee pollen that reflect the intricate relationship between bees, plants, and their environment. These compounds originate from the nectar of flowers and are influenced by various factors such flowering patterns, climate conditions, and the accessibility of nectar sources for bees. In plants, betaines—such as glycine betaine—function as osmolytes, helping to maintain osmotic balance and protect cellular structures under environmental stress. Consequently, betaine concentrations in bee pollen could serve as biochemical indicators of the environmental conditions affecting both plants and bees. Therefore, the aim of this study is to propose a novel method to determine residues in bee pollen of nine betaines and related compounds, by means of ultra-high performance liquid chromatography coupled with tandem mass spectrometry (UHPLC-MS/MS).

A quick and efficient sample treatment, involving solvent extraction with an acetonitrile and water mixture (1:1, v/v), centrifugation, freezing with dry ice followed by dilution, was proposed. Chromatographic analysis (10 min) was performed by means of a core-shell hydrophilic interaction liquid chromatography (HILIC) column, and a mobile phase applied in gradient elution mode. The method was fully validated in terms of selectivity, detection and quantification limits, matrix effect, linearity, precision and accuracy. Results showed that not only was it selective, but it also displayed a wide linearity range and good precision. Several bee pollen samples from different botanical origins were analyzed with the proposed methodology, and residues of some of the studied compounds were detected in all of them in a wide range of concentrations.

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P5. ENHANCEMENT OF THE RAMAN SIGNAL IN PRESENCE OF ANIONS

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Keywords: *EC-SERS, Electrochemistry, Raman*

The combination of Surface Enhanced Raman Spectroscopy and Electrochemistry (EC-SERS) yields a real improvement in the detection and determination of a number of different molecules.[1] Additionally, the combination of these techniques allows the discovery of new interesting phenomena, such as Electrochemical Surface Oxidation Enhanced Raman Scattering (EC-SOERS), a phenomenon reported by our group that revealed an unexpected Raman enhancement on various dielectric/semiconductors nanostructures, such as AgCl or CuI nanocrystals.

The combination of Raman with UV/Vis absorption and photoluminiscence spectroelectrochemistry allowed us to understand the origin of the enhancement of the Raman signal obtained in EC-SOERS.[2] This phenomenon can be explained in terms of the adsorption of the molecules activated by adsorbed metal cations on the dielectric/semiconductor nanocrystals,[2] generating an electrostatic interaction between analytes and substrates. The role of the adsorbed cations allows the detection of an important number of molecules, which are usually negatively charged which facilitates the electrostatic interaction with the metal cations. Therefore, the electrolytic medium plays a fundamental role in the enhancement of the Raman signal.

Unfortunately, so far EC-SOERS strategy was not able to provide Raman enhancement for positive charged molecules. However, new results suggest that these molecules can be studied by facilitating the interaction between substrate and analyte. The enhancement of the Raman signal is possible on this type of nanocrystals by playing with the electrolytic composition of the solution around the SOERS substrate. Particularly, anions are demonstrated to be fundamental to control the electrostatic attraction between the target molecules and the nanostructures generated on the rough electrode surface.

In this work, different nanostructured SOERS substrates are electrochemically generated using cyclic voltammetry, and the Raman detection is performed by using a solution containing the target molecule and a suitable electrolytic composition to enhance the Raman signal.

Acknowledgements

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P6. ADVANCED ANALYTICAL APPROACHES FOR SELENOPROTEINS AND MICROBIOTA PROFILING IN HUMAN MILK

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Keywords: *Selenoprotein P, Breast milk, ICP-MS, Microbiota profiling, Trace element speciation*

Herein, an analytical approach has been developed for the absolute quantification of selenoproteins in HM. This method is based on a novel two-dimensional column-switching system integrating three serially connected size exclusion chromatography (SEC) columns and one affinity column, hyphenated to inductively coupled plasma mass spectrometry (ICP-MS). This setup enabled precise quantification of selenoproteins and selenometabolites by species-unspecific isotopic dilution ICP-MS. Among other selenoproteins previously reported in HM, selenoprotein P was identified in HM by combining SEC-ICP-MS with bottom-up proteomics. Thus, structural confirmation and identification of Se-containing peptides, including selenocysteine (SeCys)-bearing fragments, were achieved via high-resolution quadrupole time-of-flight mass spectrometry (Q-TOF-MS) after tryptic digestion. Our results reveal that SELENOP is a significant Se species in colostrum, with an average concentration of 20.1 ± 1.0 ng Se g^{-1}, representing 31% of total Se. The Se speciation profile followed the order: glutathione peroxidase (GPX) ≈ SELENOP > selenocystamine (SeCA) > other selenometabolites (SeMB), challenging previous reports that pointed out higher levels of SeCA than SELENOP [1,2].

In parallel, meta-taxomomics was combined with our methodology to obtain the microbiota profile in HM by 16S rRNA gene amplicon sequencing using the Illumina platform. Interestingly, several associations were found between selenoproteins and microbes in HM and in the newborn microbiota. Samples with higher levels of selenoproteins exhibited distinct microbial community structures compared to those with lower selenoprotein concentrations. These preliminary observations suggest potential associations between Se speciation—particularly SELENOP abundance—and the composition of HM microbiota, opening new avenues to explore the role of trace element metabolism in shaping early-life microbial ecosystems.

This study underscores the power of multidimensional chromatographic systems coupled to ICP-MS and Q-TOF-MS in the accurate characterization of trace-level metalloproteins in complex biological matrices, while also integrating microbiological analysis to provide a holistic view of maternal-infant health.

Acknowledgements

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P7. DETERMINATION OF DELTAMETHRIN AND ITS METABOLITES IN PINE-DERIVED MATRICES

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Keywords: *deltamethrin, green analytical chemistry, gas-chromatography, method development and validation, pine needle, pine nut.*

Pine nuts and pine needles from *Pinus pinea L.* are essential components of both the Mediterranean ecosystem and agricultural industry. However, these valuable resources are threatened by the Western conifer seed bug (*Leptoglossus occidentalis*), a pest that damages pines by feeding on their vegetative and reproductive structures. This insect is particularly harmful as it destroys cones at different developmental stages, preventing them from reaching maturity. To mitigate infestations, pesticides like deltamethrin are commonly applied in pine forests. While effective against pests, their widespread use raises significant concerns regarding environmental pollution and food safety. Moreover, deltamethrin may undergo degradation, forming metabolites such as 3-phenoxybenzoic acid and 3-(2,2-dibromoethenyl)-2,2-dimethylcyclopropanecarboxylic acid, which require further investigation to understand their persistence, degradation pathways, and potential toxicological implications in comparison to the parent compound.

This study presents the development of an analytical methodology for detecting deltamethrin and its primary metabolites in pine needle and pine nut samples. The method utilizes gas chromatography coupled to quadrupole time-of-flight mass spectrometry. Two distinct sample preparation techniques were optimized: solvent extraction for pine needles and a modified QuEChERS (Quick, Easy, Cheap, Effective, Rugged & Safe) approach for pine nuts, both followed by a clean-up step prior to analysis (see **Figure 1**).

The method was assessed using the Modified Green Analytical Procedure Index (MoGAPI) and the Blue Applicability Grade Index (BAGI), confirming its environmental friendliness (greenness) and practicality (blueness) respectively. Method validation included selectivity, detection limits ranging from 0.2 to 26 μg kg^{-1}, quantification limits between 0.6 and 79 μg kg^{-1}, matrix effects (< ± 20%), recovery (82-102%), and precision (relative standard deviation < 9%). Finally, the analysis of twenty pine samples collected from Spain showed no detectable residues of deltamethrin or its metabolites.

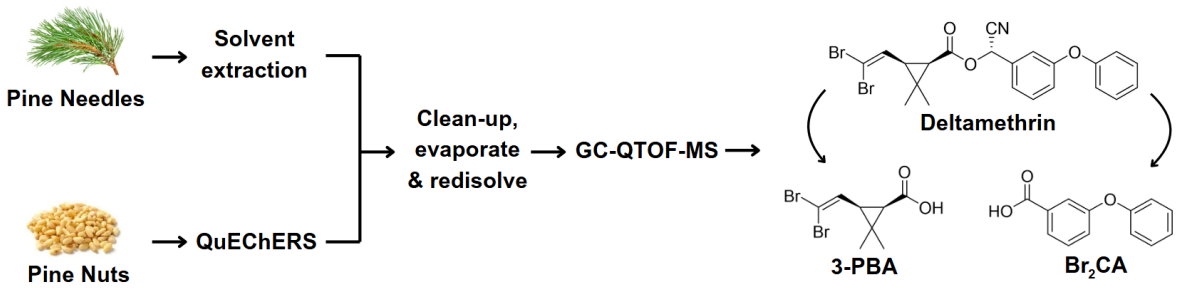

Figure 1. Analytical procedure for determining deltamethrin and its metabolites in pine matrices

Acknowledgements

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P8. SAMPLE PRETREATMENT STRATEGIES FOR NP ASSESSMENT IN HUMAN URINE AND SERUM BY spICP-MS

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Keywords: *silver nanoparticles, titanium dioxide nanoparticles, human body fluids, sample pretreatment, spICP-MS*

The spread use of inorganic nanoparticles (NPs) in various human-related sectors particularly in nanomedicine, cosmetics, and the food industry, raises concerns about their potential toxicological impact on biological systems. Nonetheless, NP assessment in human body fluids is quite a challenge. NP interactions within a biological environment as well as the very low concentration expected are key parameters. Overcoming these limitations requires advanced characterization/quantification analytical methodologies and effective sample pretreatment protocols.

The present study provides a comprehensive evaluation of several methodologies for NP isolation/preconcentration from human urine and serum: surfactant-assisted dispersive liquid-liquid microextraction (SADLLME), enzymatic hydrolysis, and centrifugal ultrafiltration (UF), selected for their potential to enhance NP recovery and maintain NP integrity. In the SADLLME approach, Triton X-114 was utilized as the surfactant to facilitate mass transfer and effective NP extraction. For enzymatic hydrolysis, a pancreatin-lipase enzyme mixture (0.1 % (w/v) each one) operating at physiological pH (7.4) and a controlled temperature of 37 ºC was selected. The enzymatic digestion was conducted under orbital – horizontal shaking to ensure homogeneous mixing and optimal enzymatic activity. For the UF approach, membranes with a molecular weight cutoff (MWCO) of 30 kDa were selected to preconcentrate NPs based on size exclusion.

Following NP extraction, single particle inductively coupled plasma mass spectrometry (spICP-MS) analyses were conducted. Instrumental and data acquisition parameters were selected appropriately to ensure sensitive detection by monitoring ^{49}Ti and ^{107}Ag signals. Experimental parameters including volume and concentration of reagents, extraction time, vortexing, or centrifugation settings, were thoroughly investigated and optimized. Under optimum conditions, analytical characteristics were assessed for each strategy proposed. Moreover, particular emphasis was placed on the determination of NP stability throughout the extraction processes by assessing size distribution profiles.

The feasibility of the proposed strategies for NP extraction and preconcentration was discussed. The discussion includes a critical evaluation highlighting the strengths and weaknesses of each technique on both extraction efficiency and the preservation of NP structural integrity.

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P9. LOW-COST NEAR-INFRARED SENSOR TO DETECT MILK ADULTERATION

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Keywords: *near infrared spectroscopy, food fraud detection, low-cost devices*

Near infrared spectroscopy (NIRS) is a well-established technology for food fraud detection and its use is becoming more and more widespread. One of the most recent trends is the implementation of monitoring systems on the production line directly, instead of analysis in the laboratory, as it is faster, and more samples can be analysed. Milk powder is highly susceptible to adulteration, second in prevalence only to olive oil [1]. One of the simplest methods of adulterating this product is the addition of odorless and tasteless substances, such as plant-based flours (e.g., rice or soybean). This practice not only raises the risk of contamination or unexpected allergic reactions but also alters the nutritional value of the final product.

The aim of this work is to evaluate a system for the detection of food fraud in powdered milk using a low-cost NIR spectrometer (Texas Instruments' NIRscan Nano evaluation module (EVM) spectrometer) [2] equipped with a fibre optic probe.

A set of 174 samples were prepared from three powdered milks brands adulterated with different percentages (2-20%) of vegetable flours: corn, soybean and rice. For the development of the system, optimisations of the measurement mode and spectral reproducibility were carried out. Different mathematical pre-treatments such as light scattering correction (SNV) or smoothing were evaluated in order to obtain the best qualitative and quantitative results. For the qualitative analysis, the ability to discriminate between types of milk powders and types of adulterants was tested using unsupervised methods such as principal component analysis (PCA) and for the quantitative analyses, regressions were carried out, using partial least squares (PLS) regression in order to evaluate the ability to quantify total adulteration in milk powders.

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P10. NEW STRATEGIES FOR THE DETECTION OF ANTICANCER DRUGS USED FOR CHRONIC MYELOID LEUKEMIA IN BIOLOGICAL FLUIDS USING NANOMATERIALS

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Keywords: Chronic myeloid leukemia, Imatinib, Nilotinib, Fluorescence, Graphene Quantum dots, Silver nanoparticles.

Chronic myeloid leukemia (CML) is a hematological disease characterized by the presence of the Philadelphia chromosome, resulting from the genetic translocation between chromosomes 9 and 22. This change gives rise to the BCR-ABL gene, which produces an abnormal protein responsible for the uncontrolled proliferation of cells in the bone marrow [1].

Thanks to this discovery in 1973, advancements in treatment have been significant. Today, tyrosine kinase inhibitors (TKIs), such as imatinib, can cause significant side effects in patients. Therefore, it is essential to develop analytical methods that allow the detection of these drugs in biological samples and monitor their concentration in the body, thereby improving disease management. In this regard, by using nanomaterials due to their exceptional properties, we have developed a spectrofluorometric method that will enable us to detect the drug over a wide concentration range (0.01-0.25 mg/L) and significantly improve the sensitivity and selectivity achieved by other methods. The selected nanomaterial was GQDs, and the recovery values obtained in biological fluids ranged between 95% and 105%

In some cases, imatinib treatment loses effectiveness, making it necessary to switch to Tasigna, whose active ingredient is nilotinib. With the same goal of optimizing drug detection and monitoring, we developed a method similar to the previous one but specifically adapted for this compound. To achieve this, we selected silver nanoparticles, a nanomaterial that offers better compatibility with nilotinib. Additionally, we obtained a calibration curve covering a wide range of concentrations, enhancing the precision and reliability of the analysis. This method will be applied to different biological samples such as serum and urine, and it has provided high recovery values (97-102%).

These studies have allowed the detection of nilotinib and imatinib using an optimized fluorescent technique, combined with nanomaterials that improve the analytical response. To achieve this, the chemical and instrumental conditions were carefully adjusted to maximize the sensitivity of the method.

Since the biological samples have complex matrices, it was necessary to implement a solid-phase extraction process, thus guaranteeing an efficient separation of the compounds of interest. The proposed methods have been successfully applied to hospital samples, demonstrating high precision and accuracy in the quantification of these drugs at the levels required for clinical monitoring.

Acknowledgements
The Spanish ministry of science and Innovation, Junta de Comunidades of Castilla-La Mancha and University of Castilla-La Mancha for financial support through the projects PID2022-138761NBI00, SBPLY/21/180501/000188, SBPLY/23/180225/000153 and 2022- GRIN-34376, SBPLY/23/180225/000153, respectively.

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P11. SUSTAINABLE PRODUCTION OF BIOACTIVE FULL EXTRACTS FROM CITRUS PEELS USING SUPRAMOLECULAR BIOSOLVENTS BASED ON POLYOXYETHYLENE SORBITAN MONOSTEARATE

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Keywords: *Bioactive compounds, orange peel residues, Supramolecular solvents.*

Worldwide citrus production is estimated to reach 173.8 million metric tons by 2026. About one-fifth of the citrus fruit grown worldwide is used for production of juices. In this process, only around 45% of the total citrus weight is converted in juice whereas the rest is wasted. Orange peel has been traditionally considered a low-value by-product of the citrus industry. However, it has recently gained prominence due to its remarkable richness in bioactive compounds, which have been shown to have recognized health benefits. Major bioactives in orange peel includes flavonoids such as hesperidin and naringin, phenolic acids, essential oils, and carotenoids.

In the context of growing demand for natural ingredients, the search for sustainable production of innovative bioactive-rich formulations has become increasingly challenging. In this respect, the production of bioactive full extracts, which contain all the bioactive compounds from a plant or other source, are highly promising since there is a broad consensus that antioxidants interact in vivo and consequently is their combination or mixture effects what leads to health benefits. Several mechanisms have been reported for such interactions (e.g. recycling of a specific antioxidant by another one after scavenging of the free radical, use of different pathways for radical inhibition, etc.) and many positive synergistic effects related to the antioxidant capacity of mixtures of hydrophilic and lipophilic compounds have been described. So, in order to fulfil the growing demand for bioactive full extracts, extraction methods for phytochemicals from biomass should be able to simultaneously extract both hydrophilic and lipophilic antioxidants in a cost-effective and eco-friendly way. Simultaneous extraction of both types of antioxidants from biomass using organic solvents is inefficient because extraction yields for these phytochemicals are highly dependent on the polarity of the solvent.

In this study, we propose the use of a new supramolecular biosolvent (bioSUPRAS) for the simultaneous extraction of a wide polarity range of antioxidants in order to produce bioactive full extract formulations. The bioSUPRAS was synthesised through the controlled self-assembly and coacervation of polyoxyethylene sorbitan monolaurate (Tween 20) in aqueous media, induced by the presence of either sulfate or citrate ions. Both hydrophilic and lipophilic antioxidants were extracted from orange peels. The extraction conditions, including the biosolvent composition, the solvent-to-sample ratio, and the extraction time, were optimized based on the total content of polyphenols and carotenoids recovered. The extracted compounds were identified and quantified using a combination of liquid chromatography and tandem mass spectrometry.

A comparison of the Tween 20-based bioSUPRAS with traditional solvents revealed superior efficiency, a more extensive recovery of bioactive compounds, a markedly reduced solvent consumption, and more gentle extraction conditions.

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P12. DETERMINATION OF VANCOMYCIN USING FLUORESCENCE SPECTROSCOPY

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Keywords: *Fluorescence, Vancomycin, antibiotic*

The inadequate dosing of medication administered to hospital patients, is currently resulting in several challenges due to the incomplete metabolism of these drugs by the body. Consequently, patients excrete them in their original form, which subsequently enters the water system and accumulates in rivers, potentially causing harm to the ecosystem. For instance, these substances can be absorbed by wildlife and plants, resulting in significant issues with bacterial resistance. Furthermore, the excessive use of antibiotics can be highly toxic to patients.

This work aims to develop a method for the rapid and straightforward quantification of vancomycin utilizing fluorescence spectroscopy. Vancomycin [1] is an antibiotic of natural origin which is listed on the model lists of essential medicines by the World Health Organization due to its effective antimicrobial action against Gram-positive bacteria, and especially for being effective against methicillin- and penicillin-resistant species, such as *Staphylococcus aureus*, or against other bacteria resistant to other antibiotics, such as *Enterococci* and *Staphylococci*. Additionally, vancomycin can be administered to patients allergic to other antibiotics, such as β-lactams.

The objective of this communication is to implement this method to ensure therapeutic monitoring of the drug, thereby preventing bacterial resistance and patient toxicity, as well as assessing vancomycin's environmental accumulation and exploring potential solutions.

In the course of the present study, it was discovered by using UV/Vis absorption and fluorescence spectroelectrochemistry that vancomycin is subject to photodegradation when exposed to ultraviolet light for a specific duration. Moreover, the photodegradation product was observed to emit fluorescence at 345 nm. Figure 1 shows the evolution of the fluorescence at 345 nm during the photodegradation of vancomycin at different concentrations.

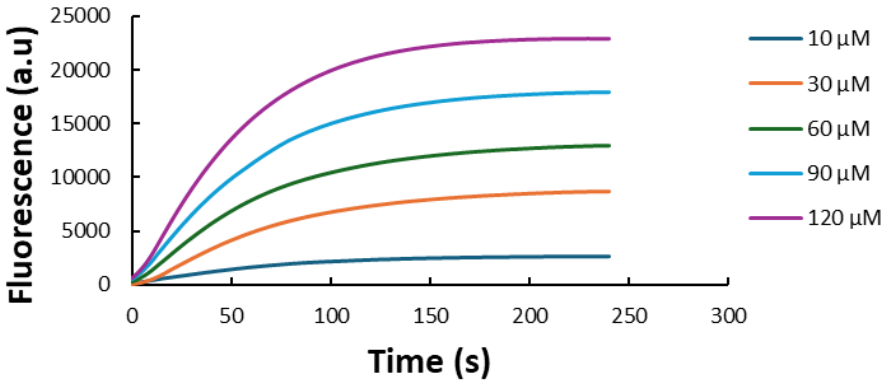

Figure 1. Evolution of the fluorescence at 345 nm with time at different vancomycin concentrations.

A method was developed for the determination of vancomycin based on its photodegradation. This method underwent optimization of various aspects, including the LED for excitation of vancomycin, the pH of the electrolytic medium, or the molecule's photodegradation time.

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P13. UNTARGETED METABOLOMICS, METALLOMICS AND HETEROATOM TAGGED PROTEOMICS REVEALED ALTERATIONS IN GUT METABOLITES, METALS AND SELENOPROTEINS IN MICROBIOTA DEPLETED MICE AFTER SELENIUM SUPPLEMENTATION

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Keywords: *selenium supplementation, metabolomics, metals, selenoproteins, gut microbiota, mass spectrometry, inductively coupled plasma, quadrupole-time of flight, mice.*

Selenium (Se) is an essential trace element with important health benefits due to the antioxidant properties of selenoproteins [1]. To analyze the interplay between Se and gut microbiota, gut metabolomic profiles, microbes, and selenoproteins were determined in the gut content of conventional (C) and microbiota-depleted mice (Abx) after Se supplementation (Abx-Se). Microbially-produced metabolites were analyzed by untargeted metabolomics, using an analytical multiplatform based on GC-MS and UHPLC-QTOFMS. Gut microbiota profiling was performed by 16S rRNA gene amplicon sequencing using Illumina. Finally, selenoproteins in mice serum were determined by heteroatom-tagged proteomics. Significant differences in the levels of about 70% of the gut metabolites determined, including fatty acyls, glycerolipids, glycerophospholipids, and steroids, were found in Abx-Se compared to Abx, and only 30% were different between Abx-Se and C, suggesting an important effect of Se-supplementation on Abx mice model metabolism. At genus level, the correlation analysis showed strong associations between metabolites and gut bacterial profiles. Likewise, higher abundance of *Lactobacillus spp.*, a potentially beneficial genus enriched after Se-supplementation, was associated with higher levels of prenol lipids, phosphatidylglycerols (C-Se), steroids and diterpenoids (Abx-Se), and also with lower levels of fatty acids (Abx-Se)[2]. Thus, although further studies to clarify the specific mechanisms are needed, we observed a crucial interaction between Se intake−microbiota−metabolites,. Additionally, Se-supplementation modulated the concentration of the antioxidant glutathione peroxidase (eGPx) and the Se-transporter selenoalbumin (SeAlb) as well as the metal homeostasis, being influenced by microbiota disruption, which suggests an intertwined mechanism[3]. Se also modulated microbiota diversity and richness and increased the relative abundance of some health-relevant taxa (*e.g.*, families *Christensenellaceae*, *Ruminococcaceae*, and *Lactobacillus* genus). This study demonstrated the potential beneficial effects of Se on gut microbiota, especially after antibiotic treatment, and the first associations between specific bacteria and plasma selenoproteins and gut metabolites.

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P14. PROMOTING THE EXTRACTION OF PROTEINS FROM HOUSE CRICKET (*ACHETA DOMESTICUS*) BY PRESSURIZED LIQUIDS EXTRACTION AS AN ALTERNATIVE TO CONVENTIONAL METHODOLOGIES

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Keywords: *proteins, edible insects, sustainable techniques.*

The use of edible insects as an alternative protein source has attracted growing interest, particularly after the European Union authorized their consumption, recognizing their safety and nutritional value. Among these, *Acheta domesticus* (house cricket), approved in February 2022, stands out due to its high content of quality proteins, essential amino acids, vitamins, and minerals. Moreover, cricket farming requires significantly less water, feed, and land than conventional livestock, resulting in a lower environmental impact. These characteristics make cricket a sustainable and promising alternative to address future nutritional and ecological challenges. One way to increase the appeal of insect consumption in Western societies is by using isolated insect fractions rather than whole insects. In this regard, this fraction is highly appreciated, although not much research has been performed to explore alternatives to the conventional alkaline solid-liquid extraction. This methodology often results in low yields, long extraction times, and low-quality proteins. This works proposes the use of pressurized liquid extraction as an alternative technique for this purpose.

A Box–Behnken experimental design was employed to maximize protein recovery while minimizing the co-extraction of undesirable compounds such as chitin and the formation of Maillard reaction products. Different water–ethanol mixtures were employed observing that 100% water resulted in greater protein extraction. Both extraction time and temperature were found to positively influence the protein yield. Additional optimization strategies were explored to further enhance extraction efficiency. These included pre-extraction defatting of the insect biomass and the combination of PLE with alkaline extraction and enzyme-assisted extraction. Notably, the protein yield was significantly higher when PLE was performed in alkaline media compared to conventional alkaline extraction.

Proteins in the final extract and in the extract obtained by conventional alkaline solid-liquid extraction were characterized through sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), reversed-phase high-performance liquid chromatography (RP-HPLC) and Fourier-transform infrared spectroscopy (FTIR), the latter enabling the assessment of protein secondary structure.

P15. PHOSPHOLIPID DETERMINATION BASED ON THE FORMATION OF GOLD NANOPARTICLES

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Keywords: phospholipids, gold nanoparticles, HPTLC

The role of phospholipids (PLs) is crucial in assessing lipid degradation in food of animal origin. PLs undergo degradation processes that are influenced by storage conditions, temperature, oxygen exposure, packaging method, and cooking practices.

High-Performance Liquid Chromatography (HPLC) is currently the most widely used technique for comprehensive PL analysis. However, High-Performance Thin-Layer Chromatography (HPTLC) is gaining popularity due to its rapid separation and adequate resolution of PL families [1].

The aim of this study is to develop a methodology for monitoring PLs and their oxidation products in food using HPTLC, based on the formation of gold nanoparticles (AuNPs). PLs can act as reducing agents for Au(III), while their lipid structures serve as capping agents for the resulting nanoparticles.

The results indicate that unsaturated PLs are more likely to promote the formation of AuNPs under the following conditions: phosphate buffer (0.1 M, pH 7), Au(III) at 1 mM, and a temperature of 40 °C. Moreover, AuNP formation is enhanced as the degree of unsaturation of the PLs increases.

Figure 1 shows the correlation between the concentration of phosphatidylcholine 18:1/18:1 and the absorbance at 570 nm, both in solution and in the inset image on a C18 silica gel plate.

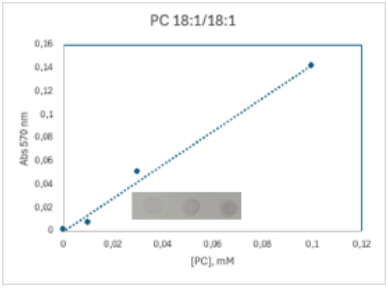

Figure 1: Variation of AuNPs maxima absorption (570nm) with the concentration phosphocholine 18:1/18:1

Future work will focus on detecting various unsaturated PLs on HPTLC plates and further optimizing the experimental conditions for the detection of saturated PLs.

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P16. USE OF BIMETALLIC NANOPARTICLES AS NOVEL TAGS IN LATERAL FLOW IMMUNOASSAYS FOR CHRONIC WOUND BIOMARKER DETECTION

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Keywords: *lateral flow*, *bimetallic nanoparticles, biomarkers, chronic wound*

In recent years, the use of lateral flow immunoassays (LFIAs) has stood out as a basic tool for monitoring disease status in the population, as in the case of COVID-19 pandemic, being extended for point-of-care (POC) diagnostics. The low cost, portability, simplicity and rapid detection by naked eye make LFIAs one of the basic tools in the clinical biosensing field. However, there are some drawbacks mostly related to the low sensitivity of such assays. Different approaches have been evaluated to improve sensitivity, mainly based on the introduction of new nanomaterials or modifications of the pad architecture to modify the flow rate [1].

One of the main factors regarding the sensitivity of lateral flow immunoassays (LFIAs) is related to the selection of nanoparticles used as detection tags, which are conjugated with specific antibodies, responsible for the appearance of the positive detection line in the strip. Gold nanoparticles have become the most widely stablished choice due to their unique optical properties, ease of synthesis and conjugation with biological receptors. However, in certain cases gold nanoparticles exhibit a relatively low optical signal compromising the sensitivity of the assay. Additionally, to prevent non-specific adsorptions at the test line, an extra blocking step is sometimes required, adding complexity to the assay preparation.

In this context, we propose the use of nanoraspberry-shaped PtAu bimetallic nanoparticle tags as novel and advantageous alternative to the standard AuNPs. Such nanoparticles have been previously used in electrocatalytic [2] and electrochemiluminescence [3]-based approaches but have not been exploited yet in LFIAs. The improved performance expected in LFIA is related to: i) an enhanced nanoparticle/antibody ratio thanks to the morphology of the NPs, in particular to the Au protuberances grown in the platinum surface and ii) the intense purple color which can provide a better contrast with the white background of the nitrocellulose membrane.

The proposed methodology is applied for the detection of myeloperoxidase, a key biomarker in the management of chronic wounds, as it allows monitoring of both infection and healing processes. Specific biomarkers present in wound exudates serve as valuable tools for this purpose as their presence and levels are reliable indicators of the physiological state. In particular, the levels of some human-secreted enzymes such as myeloperoxidase can reflect the status of infection and tissue regeneration status. Therefore, there is a clear need for the development of rapid, user-friendly analytical tools for the on-site detection of biomarkers associated with chronic wound infection and healing.

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P17. NEXT GENERATION SEROLOGY: IMMUNIZATION STATUS PROFILING VIA ELECTROCHEMICAL DNA SCAFFOLD SENSORS

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Keywords: *DNA scaffold sensors, serologic profiling, infectious diseases, point of need*

Vaccine-preventable diseases exhibit a global resurgence due to suboptimal vaccine coverage. One of the main reasons acknowledged by the World Health Organization is the increasing trend of international travel. Some studies[1] suggest that consistent serological testing can identify non-immune travellers in a cost-effective way. However, this strategy might not be suitable for last-minute travellers or infectious diseases requiring multiple vaccine doses. Therefore, serological assessment at the point of care (PoC) during the travel would help circumvent current challenges, while it also would signify a step towards personalized medicine.

Classic laboratory techniques such as Enzyme-Linked Immuno-Sorbent Assay (ELISA), despite their efficacy, require long incubation times, multiple manipulation steps, specialized equipment and trained experts to be carried out. Other techniques more suitable for a PoC application, such as Lateral Flow Assays, although fast and easy to use, are difficult to make compatible with multiplex and quantitative detection.[2]

In this context, the SerDNA project aims to develop rapid diagnostic tests that can efficiently detect the presence of clinically relevant antibodies at the PoC. Namely, antibodies against dengue, hepatitis A and measles viruses antigens. In order to circumvent the aforementioned limitations, electrochemical DNA scaffold sensors are arising as an alternative. As illustrated in **Figure 1**, these sensors consist on a double stranded DNA immobilized on one end to the electrode surface, and presenting a redox label and a specific epitope on the other end. In the absence of antibody target, the DNA scaffold is free to move, and the electron transfer between redox label and electrode is unimpeded. However, the presence of antibody target and the subsequent binding to the epitope induce steric hindrance that hinders the electron transfer efficiency, studied with voltammetric techniques.

Thus, DNA scaffold sensors offer a quick, one-step approach for the quantitative detection of antibodies in low volume samples.[3] Their compatibility with PoC testing makes them ideal for taking the diagnosis and management of vaccine-preventable infectious diseases a step forward.

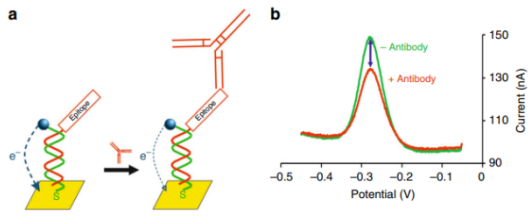

Figure 1. Structure and principle of operation of electrochemical DNA scaffold sensors. (a) In the absence of the targeted antibody, the DNA scaffold moves freely and electron transfer is unimpeded. Upon antibody binding, steric hindrance interferes electron transfer. (b) Changes in electron transfer rate can be quantified by means of electrochemical techniques such as square wave voltammetry.[2]

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P18. DEVELOPMENT OF NEW STRATEGIES FOR THE ANALYSIS OF BACTERIA

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Keywords: *Bacteria, EC-SERS, Raman, Electrochemistry*

The detection, identification, and quantification of microorganisms have become critical concerns in areas such as food and water safety, biodefense, human health, and diagnostics. The prevailing standard for bacterial identification relies on culturing bacteria and analyzing their morphological and metabolic characteristics. The development of alternative methods for bacterial identification is of significant interest to society, since a rapid and sensitive detection of bacteria could accelerate diagnosis from several days to a few hours. In this context, Surface Enhanced Raman Spectroscopy (SERS) emerges as a promising technique for the detection and identification of bacteria, thanks to its remarkable sensitivity and the unique molecular fingerprint it offers. In the literature, bacteria have been investigated using SERS[1] and electrochemical-SERS (EC-SERS)[2], employing various analytical strategies, typically based on SERS substrates derived from chemically synthesized plasmonic nanostructures. An interesting alternative to chemical synthesis is the electrochemical synthesis of nanostructures. EC-SERS[3] enables the preparation and detection of target molecules in a single experiment. SERS substrates developed on screen-printed electrodes (SPEs) have proven to be highly effective in generating sensitive and reproducible platforms for Raman analysis. Furthermore, the molecular fingerprint of target molecules evolves with potential, offering valuable insights for their detection.

Most SERS techniques for bacterial identification rely on attaching SERS tags to the bacterial membrane. While this approach is highly reliable, it is based on intricate procedures to achieve optimal results. We suggest inducing the release of small molecules from the bacteria through chemical methods. Once these molecules are released, an EC-SERS experiment can capture a unique fingerprint of the bacteria based on the detected molecules. By using various chemical reagents to trigger the release of these molecules, different Raman spectra can be produced. In this study, we propose several strategies to achieve the most accurate Raman fingerprint by controlling the chemical reagents used to release the bacteria's characteristic molecules and employing different EC-SERS techniques for the detection step.

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P19. ENHANCED EXTRACTION OF BIOACTIVE ESSENTIAL OILS AND POLYPHENOLS FROM AVOCADO RESIDUES: CHEMICAL PROFILING AND BIOACTIVITY ASSESSMENT

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Keywords: *avocado by-products, circular economy, extraction optimization, polyphenols, terpenes, antioxidant capacity*

The increasing demand for fine, natural, and eco-friendly chemicals, along with the need for sustainable growth, underscores the importance of optimizing the use of food industry residues. Among these, avocado (*Persea americana* Mill.) waste has gained significant attention due to its substantial consumption, which has tripled over the past 20 years. This surge in avocado production, resulting in approximately 30% of the fruit being discarded as waste, primarily peels and seeds, presents a serious challenge. Avocado residues are rich in valuable compounds such as monounsaturated fatty acids, tocopherols, polyphenols, and terpenes. Therefore, the valorization of avocado waste enables the recovery of high-value-added and bioactive compounds, addressing both the environmental issues associated with waste management, and the growing demand for functional and natural products [1,2]. Consequently, it is crucial to develop solvent-based extraction strategies that are simple, fast, environmentally friendly, and adaptable for industrial use.

This study proposes the recovery of valuable bioactive fractions from avocado seeds and peels, specifically focusing on volatile essential oils enriched with terpenes and hydrophilic fractions enriched with phenolic compounds. To achieve this, a green ultrasound-assisted extraction (UAE) method was developed and optimized for recovering the phenolic fraction from avocado peels. This was accomplished using a three-level, two-factor factorial experimental design, evaluating the ethanol-water proportion (0:100–80:20 %, v/v) and extraction time (5–25 min), after defining the probe amplitude (80%) through prior screening factorial design. The evaluated responses included total phenolic content, total flavonoid content, and DPPH antioxidant activity. Once the optimal UAE conditions were established, the phenolic extract was analyzed using cLC-DAD-ESI-MS, revealing the presence of gallic acid, quercetin, and kaempferol. Additionally, *in vitro* lipid peroxidation inhibition and antibacterial activity were evaluated, along with the greenness of the UAE method using the AGREEprep tool. For the volatile fraction, essential oils from avocado seeds were extracted by hydrodistillation and analyzed by GC-MS, with the sesquiterpene fraction being predominant. The antioxidant and antibacterial activity of this fraction was also assessed using the disc microdiffusion method and the DPPH assay.

In conclusion, the proposed approach is simple and efficient to achieve a holistic valorization of avocado waste. It promotes the sustainable recovery of diverse bioactive compounds, which have potential applications in the cosmetic and agri-food industries as aromatic, antibacterial and antioxidant additives.

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P20. STIR BAR SORPTIVE DISPERSIVE MICROEXTRACTION FOR THE DETERMINATION OF MIFEPRISTONE IN WATER

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Keywords: *mifepristone, embedded magnetic nanoparticles.*

The discharge of emerging contaminants to the environment has led to a major focus on their research. This is the case of Mifepristone (MIF), an abortive drug with a high risk to wildlife due to its properties as endocrine disruptor. From the analytical point of view, sensitive and selective analytical techniques are essential for the detection and quantification of this compound, particularly in water bodies. Liquid chromatography (LC) coupled to different detectors meets these criteria and has been used for this type of analysis; however, sample preparation for the clean-up and/or concentration is still required.

Solid-phase extraction (SPE) dominates the field of sample preparation, but in the last decades, new operational aspects have been incorporated to SPE, resulting in new techniques, such as stir bar sorptive dispersive microextraction (SBSDME), and new (nano)materials have been incorporated as sorbents [1]. In this work, SBSDME with magnetic nanoparticles of cobalt ferrite ($CoFe_2O_4$) embedded in a commercial styrene-polivinylbenzene sorbent (EBH^{TM}) has been used for sample preparation prior to the determination of MIF by LC and UV detection.

The sorbent was characterized for size, magnetism, and zeta-potential. The extraction method and the LC conditions were optimized in a univariate way. The full method was validated achieving a detection limit of 0.36 µg/L, an enrichment factor of 35.7, and a reusability of the same sorbent up to 3 times. It was applied to samples obtained from the Tagus River (Spain) with different content of salts and organic matter, with relative recoveries close to 100 %. The method was considered green by both the AGREE and AGREEprep metrics [2,3].

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P21. POSTHARVEST LIGHT APPLICATION ENHANCES HEALTH-PROMOTING COMPOUNDS IN MINIMALLY PROCESSED BABY LEAF LETTUCE

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Keywords: *Lactuca sativa L., Ready-to eat salads, Bioactive compounds, Health benefits, Phenolic compounds, Antioxidant activity.*

Lettuce is one of the most popular leafy greens on tables around the world, appreciated not just for its crisp bite and mild flavor, but also for the health benefits it brings. As modern lifestyles continue to favor foods that are fresh, quick, and easy to prepare, lettuce has become a go-to ingredient in ready-to-eat meals, especially in salads. Among the different types, baby leaf lettuce stands out. Its tender leaves, appealing look, and shorter growing time make it a favorite for both producers and consumers. But lettuce isn't just about convenience, it's also packed with nutrients. Lettuce is rich in compounds like polyphenols, vitamin C, vitamin E, and carotenoids, all known for their antioxidant properties that help protect the body against disease. The growing popularity of baby lettuce highlights not just changing eating habits, but also a greater interest in foods that support a healthy lifestyle.

Light is one of the most important environmental factors that can affect phytochemical concentrations in plants. The aim of this work was to evaluate the effect of postharvest LED treatment on improving health-promoting compounds, such as chlorophylls, carotenoids, and phenolic compounds, in nine cultivars of minimally processed baby leaf lettuce.

This research demonstrated considerable variability in the content of health-promoting compounds among different types of baby leaf lettuce. Using LC-MS, 28 polyphenolic compounds were identified, with notable differences in their profiles across the various cultivars. Chicoric and chlorogenic acids were found in the highest concentrations and showed a strong association with antioxidant activity, whereas pigment levels did not correlate with this property. Notably, LED light treatment emerged as a promising strategy to enhance the accumulation of these bioactive compounds. Among the cultivars studied, Milly and Paris Island (representing Green Butterhead and Romaine types, respectively) and Grenadine (a red Oak Leaf type) stood out for their superior health-promoting potential. These insights can help drive consumer demand and facilitate the introduction of healthier baby leaf lettuce options into the market.

P22. USE OF CARBON DOTS AS A SENSOR MATERIAL FOR THE DETERMINATION OF TITANIUM NANOPARTICLES IN WATER SOURCES

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Keywords: *carbon dots, fluorescent, photocatalysis*

Inorganic nanoparticles (NPs) are present in a wide range of products, particularly cosmetics, food and medical devices, and today there is some concern about how these NPs are released into the environment. [1] In addition to improvements in nanometrology techniques for the determination/characterisation of NPs, it is important to develop rapid analytical, low cost and in situ methods. This is the goal we are currently pursuing. Carbon Dots (CDs) are a very suitable nanomaterial for the development of an in-situ sensor due to their excellent optical behaviour (luminescence properties), low toxicity and they can be obtained by several simple synthesis routes. [2, 3]

In this work, we will investigate the possibility of using CDs as a sensor material for the detection of titanium dioxide nanoparticles (TiO_2 NPs) in water sources. The methodology will be inspired by the photocatalytic properties of TiO_2 NPs. We will register the decrease in fluorescence of CDs due to their photodegradation in the presence of TiO_2 NPs. By plotting this decrease against the concentration of NPs, we obtain a linear response up to 750 µg/L.

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P23. DEVELOPMENT OF A PHOTOLYSIS-BASED METHOD FOR THE QUANTIFICATION OF NITROSAMINES USING OPTICAL SENSING

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Keywords: *nitrosamines, photolysis, reactive strip, colorimetric detection*

Nitrosamines (NAs) are compounds classified in group B2 as probable human carcinogens. Their toxicity is linked to metabolic degradation by cytochrome P450, which generates diazonium ions capable of forming DNA adducts, thereby altering its structure and function. Among the most relevant NAs are nitrosopyrrolidine (NO-Pyr) and nitrosopiperidine (NO-Pip). While their natural occurrence is already concerning, food preservation processes can significantly increase their concentration. This is largely due to the widespread use of nitrite as a preservative in the food industry to inhibit microbial growth.

A photolytic method has been developed for the decomposition of nitrosamines both in solution and on reactive strips, using different light sources (Xe lamp and LEDs of different wavelengths). From the photolysis products, two strategies were explored: (1) the formation of gold nanoparticles (AuNPs), which showed limited sensitivity, a narrow linear range, and poor nanoparticle stability; and (2) the detection of nitrite ions as a photodegradation by-product. Promising results were obtained using the Griess reaction and, in particular, fluorescence quenching with gold nanoclusters (AuNCs) with limits of detection below 0.1µM.

Additionally, a device was designed to allow both irradiation and subsequent measurement of the reactive strips. Key parameters such as exposure time, injection volume, irradiation distance, and temperature effect were analyzed. Colorimetric analysis based on RGB or CIELAB coordinates allowed for a straightforward correlation with NA concentration.

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P24. OVERCOMING MATRIX EFFECS IN SINGLE-EVENT ICP-MASS SPECTROMETRY

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Keywords: *Single-event ICP-mass spectrometry (single-event ICP-MS), micro/nano-entities, sample introduction system, matrix effects*

The characterization of micro- and nano-scale entities is increasingly important due to their broad application range across material science, environmental monitoring, and biology.[1] Single-event ICP-mass spectrometry (single-event ICP-MS) is a powerful technique for this purpose, enabling the determination of the mass, mass distribution (and size, assuming known chemical composition), as well as number and mass concentrations. In single-event mode, a highly diluted suspension of the target entities is introduced into the plasma, and the mass spectrometer is operated in time-resolved analysis mode to detect individual signal pulses corresponding to each micro- or nano-structure.[2] However, like all ICP-MS approaches, single-event ICP-MS is not free from challenges, the most important ones being the occurrence of spectral and non-spectral interferences.

Although the theoretical basis of single-event ICP-MS was established over two decades ago and many applications are now considered routine, it is timely to revisit some of its foundational concepts.[3],[4] These include calibration strategies and methods for overcoming non-spectral interferences (i.e., matrix effects), to ensure accurate results under non-ideal conditions, such as when analyzing entities in complex media.

In this work, various sample introduction systems and strategies to mitigate matrix effects in single-event ICP-MS have been evaluated. Several matrices known to induce signal enhancement or suppression have been tested, and their effect on the characterization of diverse micro/nano-structures, such as metallic nanoparticles, microplastics, and cells, has been investigated. This fundamental study aims to improve single-event ICP-MS performance and to expand its applicability to real-world sample suspensions, which often present analytical challenges.

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P25. IMPACT OF GUT MICROBIOTA ON ARSENIC AND SELENIUM METABOLISM: ASSOCIATIONS WITH SHORT-CHAIN FATTY ACIDS, ARSENIC SPECIATION, AND SELENOPROTEIN EXPRESSION

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Keywords: *Gut Microbiota, Short-Chain Fatty Acids, Arsenic Speciation, Selenoproteins, Microbiota Profiling.*

Arsenic, a toxic metalloid, affects nearly all human organs [1], with approximately 140 million people in at least 70 countries exposed to contaminated water containing arsenic levels exceeding the World Health Organization's recommended limit of 10 micrograms per liter. Selenium, on the other hand, is an essential nutrient that plays a crucial role in various cellular processes as a component of selenoproteins and selenoenzymes. The interaction between arsenic and selenium has garnered significant research interest due to the formation of arsenic–selenium complexes through reactions with glutathione and S-adenosylmethionine [2]. This interaction is partially mediated by the gut microbiota, which influences the biotransformation of both elements.

This study explored the potential impact of the gut microbiota on the metabolism and interaction of arsenic and selenium through arsenic speciation, selenoprotein determination, and gut microbiota profiling. Additionally, the study investigated the role of short-chain fatty acids (SCFAs) and examined the associations between different species of arsenic and selenium, comparing them with microbiota composition. The findings emphasize the importance of the gut microbiota in modulating the bioavailability and excretion of these elements, affecting their accumulation and toxicity. Significant associations were also identified between specific microorganisms, metabolites such as SCFAs, and key elements, highlighting the critical role of the microbiota in metal homeostasis and plasma selenoproteome expression.

The results revealed that the gut microbiota plays a crucial role in arsenic bioaccumulation and metabolization. In mice with microbiota depletion, arsenic concentration in the serum was notably higher compared to the control group. Selenium supplementation reduced arsenic levels in mice with normal microbiota, but increased them in microbiota-depleted mice, suggesting a competition between arsenic and selenium for the microbiota. Selenium supplementation also increased selenometabolites and selenoproteins in serum, suggesting that the gut microbiota is essential for selenium metabolization and its interaction with arsenic. Microbial diversity was also impacted by arsenic and selenium exposure, particularly in the microbiota-depleted mice model.

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P26. NITROGEN AND LANTHANIDE CO-DOPED POLYMERIC CARBON DOTS: NANOTAGS FOR MULTIMODAL AND TARGETED BIOIMAGING APPLICATIONS

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Keywords: *Carbon Dots, Nanotags, Multimodal Bioimaging, HeLa Cells, NIH/3T3 Cells*

Multimodal imaging is a cutting-edge technology where the advantages of nanoparticles are currently exploited, as the combination of several imaging agents into a unique nanotag (multifunctional nanomaterials) that allows a fast disease diagnosis. An alternative for multimodal application is the use of Carbon Dots (CDs). These nanomaterials (NMs) have been rising along the last years due to its tunable properties, presenting most of them simple synthesis procedures, outstanding photostability and extremely low toxicity[1]. Many of doping strategies to obtain CDs with multimodal capabilities consist in the introduction of transition metal atoms in the nanostructure or even rare earth elements.

In this work, co-doped CDs are presented as powerful nanotags for multimodal bioimaging applications[2]. N, Gd and Yb co-doped CDs were synthesized via simple one step carbonization of PEG-400 and ammonium citrate in presence of $GdCl_3$ and $YbCl_3$.

This Co-doped CDs were exhaustively characterized showing an intense fluorescence with a quantum yield higher than 50%, multiemission and 2.5±0.3nm spherical size. Cytotoxicity assays were carried out with HeLa and NIH/3T3 cells, and our results confirmed the high biocompatibility in both, healthy and tumoral cells. After that, doped-CDs functionalized with an anti-mouse polyclonal antibody showed its potential use to carry out targeted bioimaging studies against tumoral cells by recognizing cell membrane proteins. These studies have showed the potential application of this Co- doped CDs in different bioimaging techniques such as fluorescence, Laser Ablation-ICP-MS by quantifying Gd or Y and Magnetic Resonance Imaging using these Co-doped CDs as contrast agents. Results confirmed these nanomaterials as an ideal and promising nanotool to perform multimodal bioimaging studies. Moreover, the possibility to easy functionalize this nanomaterial with recognition biomolecules, such as antibodies, provides its potential as clinical tool

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P27. EFFECT OF DEEP EUTECTIC SOLVENTS AND THEIR COMPONENTS ON THE ENANTIOMERIC SEPARATION OF TIANEPTINE BY CICLODEXTRIN-ELECTRKINETIC CHROMATOGRAPHY

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Keywords: *chiral electrokinetic chromatography, cyclodextrins, deep eutectic solvents, tianeptine.*

Chirality of pharmaceutical compounds plays a crucial role in their biological performance, as enantiomers can interact in significantly distinct ways with molecular receptors. In many cases, only one of the two mirror-image forms is responsible for the therapeutic benefit, while the other may lack efficacy or trigger harmful effects. Consequently, the separation of enantiomers is essential in pharmaceutical analysis. Electrokinetic Chromatography (EKC) has shown to be a reliable technique in this context, offering chiral discrimination with minimum sample and reagents consumption and high efficiency [1].

Nowadays, an emerging and interesting research line to enhance the efficiency of chiral separations by EKC, is the use of Deep Eutectic Solvents (DESs) as additives in the separation buffer. DESs represent a novel class of solvents composed of a hydrogen bond donor and a hydrogen bond acceptor which exhibit advantageous properties including chemical stability, low environmental impact, biodegradability, and straightforward preparation. Despite the limited exploration of DESs in the context of EKC, preliminary studies indicate that DESs have the potential to enhance chiral separations when employed in conjunction with chiral selectors such as cyclodextrins (CDs) [2,3].

The aim of this work was the development of an analytical methodology for the chiral separation of tianeptine (a racemic antidepressant), based on the use of EKC with CDs as chiral selectors and the study of the effect of different DESs as enhancers of the enantioseparation. The separation of tianeptine enantiomers was investigated at two pH levels (3.0 and 7.0) using both neutral and negatively charged CDs. Among all the CDs tested, sulfated-α-cyclodextrin at 1.5% (w/v) enabled the enantiomeric separation within 10 min achieving a resolution value of 1.8. Subsequent experiments assessed the influence of multiple DESs and their individual components, identifying tetrabutylammonium bromide (TBABr) as a key additive that, in conjunction with the selected CD, further improved the method's enantioselectivity. The validated method proved to be effective in analyzing tianeptine in pharmaceutical preparations, demonstrating its suitability for quality control applications.

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P28. Comparison of SFC-MS and UHPLC MS/MS for the Enantioseparation of Amino Acid Enantiomers

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Amino acid enantioseparation is one of the challenging areas of analytical chemistry due to its complex structure and varying behaviour in various analytical techniques. Different analytical techniques have been applied for their accurate quantification to achieve maximum performance. These developments include chiral stationary phase, various mobile phase compositions, gradient elution strategies and detection techniques. To evaluate the effectiveness of these approaches, key aspects of analytical performance include Analytical Time, Selectivity (α), Retention Factor of the Enantiomers (k), Column Efficiency (theoretical plates), Sensitivity, Detection Limits and Resolution (Rs) are considered. The performance criteria may be extended to other parameters depending on the analytical target and the analytical techniques used.

This study compares two distinct separation techniques: Supercritical Fluid Chromatography-Mass Spectrometry (SFC-MS) and Ultra-High-Performance Liquid Chromatography-Tandem Mass Spectrometry (UHPLC-MS/MS), under the same chromatographic conditions, including the use of the same chiral stationary phase and mobile phase adapted to each technique's capacity. The resulting separation performances are compared based on the sensitivity, selectivity, speed and resolution. UHPLC-MS/MS demonstrated higher sensitivity and lower detection limits than SFC-MS. Meanwhile, SFC-MS provides more efficient mobile phase usage. Interestingly, more amino acids were detected in SFC than in UHPLC-MS/MS. The last peak observed in UHPLC-MS/MS was ornithine enantiomer, eluting at 12.86 minutes, whereas for SFC-MS, proline was the last peak observed at a retention time of 14.14 minutes. This may be evidence that UHPLC-MS/MS is faster than SFC. However, the conclusion may be premature since the second enantiomer of proline was not observed in UHPLC-MS/MS. Both techniques demonstrated comparable elution orders, although some enantiomers detected by SFC-MS were not observed in UHPLC-MS/MS, and vice versa. A resolution of ≥1.8 was observed in UHPLC, whereas a resolution of ≥2.0 was observed in SFC, suggesting a comparable level of resolution between the two techniques. Strangely, only one mass for histidine was detected under the same mobile phase compositions in both techniques. Although both techniques used weak acid additives, the acidic nature of CO2 in SFC may have created a more acidic environment. In conclusion, the advantages and limitations of each technique make the analytical choice critically dependent on the analytical objectives in industries such as pharmaceuticals, food, and environmental sciences.

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P29. A µSPEed MICROEXTRACTION STRATEGY AND UHPLC-PDA ANALYSIS FOR THE DETERMINATION OF VETERINARY ANTIBIOTICS

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Keywords: *veterinary antibiotics, tetracyclines, µSPEed microextraction, environmental water, liquid chromatography*

The excessive use of antibiotics in livestock farming has led to concerns regarding their presence in food and the environment, contributing to antibiotic resistance and potential health risks [1,2]. Traditional extraction techniques, such as Solid-Liquid Extraction (SLE), Solid-Phase Extraction (SPE), and Liquid-Liquid Extraction (LLE), are widely used for their determination but often require large sample volumes and solvents, raising sustainability issues. To address these challenges, this study proposes a semi-automated micro-solid phase extraction (µSPEed) methodology for the analysis of six commonly used veterinary antibiotics—tetracycline, chlortetracycline, oxytetracycline, doxycycline, sulfamethoxazole, and trimethoprim—in environmental water samples.

The developed method significantly reduces solvent and sample consumption while improving efficiency, reproducibility, and analyst workload. The extraction process was optimized by assessing different sorbents, sample volumes, loading cycles, pH conditions, and elution solvents to maximize recovery and sensitivity. The optimal conditions were achieved using a PS/DVB-RP sorbent with three 250 µL loading cycles and two consecutive elutions with 50 µL of acidified methanol (0.1% formic acid). Coupled with ultra-high-performance liquid chromatography (UHPLC) and UV detection, this methodology enabled rapid analysis with a total chromatographic run time of 6 min. The validation results confirmed excellent linearity, precision, and satisfactory recoveries. Detection limits ranged from 0.30 to 1.23 µg L^{-1}, while quantification limits were between 0.92 and 3.73 µg L^{-1}. The proposed µSPEed/UHPLC-PDA methodology provides an efficient and reliable approach for monitoring antibiotics residues in environmental samples, offering a promising tool for wastewater analysis and environmental safety assessment.

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P30. ANALYTICAL PLATFORM TO QUANTIFY ADCS FOR CLINICAL APPLICATION

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Keywords: ADC, cancer, analysis, pharmacokinetics.

Cancer remains one of the leading public health challenges worldwide. While recent advances have significantly improved the early detection and treatment of various cancers, particularly in their initial stages, the development of targeted therapies that minimize systemic toxicity continues to be a major unmet need [1].

Antibody-drug conjugates (ADCs) have emerged as a promising therapeutic strategy in this context. ADCs are a class of biopharmaceuticals that combine the selectivity of monoclonal antibodies with the cytotoxic potency of small-molecule drugs. The antibody serves to selectively deliver the cytotoxic agent to tumor cells that express the target antigen, thereby sparing healthy tissues and reducing off-target effects [2].

However, the structural complexity and heterogeneity of ADCs represent a significant analytical challenge. Once administered, ADCs can undergo various biotransformations such as deconjugation, catabolism, or payload release, which impact their pharmacokinetic and pharmacodynamic behavior. Therefore, a comprehensive characterization of the ADC and its individual components—total antibody, conjugated antibody, and free payload—is critical for understanding its in vivo behavior and for evaluating its safety and efficacy [3].

To address this challenge, robust and orthogonal analytical methods are required. Among them, liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) and enzyme-linked immunosorbent assays (ELISA) are the most widely used. LC-MS/MS offers high specificity and sensitivity for quantifying small molecules and peptides. In contrast, ELISA is a powerful tool for selectively quantifying molecules through antigen–antibody interactions [4].

Here we present the analytical platform developed and validated for the quantification of the three major analyte fractions associated with an ADC in biological samples. LC-MS/MS was applied for the quantification of total antibody and free payload, while ELISA was used to measure the conjugated fraction.

The analytical platform developed will be applied to the pharmacokinetic evaluation of a novel ADC in a Phase I clinical trial involving cancer patients.

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P31. MOLECULARLY IMPRINTED NANOPARTICLES AS SENSING ELEMENTS FOR SARS-COV-2 SPIKE PROTEIN

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Keywords: *Imprinted nanoparticles; interdigitated electrodes; impedimetric sensors.*

Molecularly Imprinted Polymers (MIP), many times referred to as artificial or plastic antibodies, are synthetic macromolecular receptors that recognise a certain target ligand with notable affinity and selectivity, imitating the binding event of natural receptors in biological systems [1]. Considering the potential benefits of imprinted nanomaterials over natural antibodies, it was intended here to produce anti-Spike molecularly imprinted nanoparticles (MIN) as antibody substitutes able to recognise a linear 9 amino acid sequence (470-TEIYQAGST-478) of the spike protein of the SARS-CoV-2 virus. The mentioned sequence is located at the receptor-binding domain (RBD) of the virus, and more precisely at the receptor-binding motif (RBM) responsible for the binding and subsequent entry into human cells via the ACE-2 receptor [2]. Three different monomer mixtures were employed to fabricate imprinted and non-imprinted (control, NIN) nanoparticles combining the epitope imprinting and solid phase synthesis approaches.

Both MIN and NIN were characterised by Dynamic Light Scattering (DLS) and Transmission electron microscopy (TEM), finding that nanoparticles' size was 130.7 ± 9.4 nm for MIN and 125.1 ± 0.6 nm for NIN. Binding behaviour of the produced materials was examined by Surface Plasmon Resonance (SPR). In this setting, gold chips were thiolated using mercaptoundecanoic acid (MUA) with the aim of creating a carboxyl terminated SAM monolayer to later graft nanoparticles through carbodiimide chemistry (EDC/NHS). Unreacted NHS groups were deactivated using ethanolamine. Equilibrium dissociation constant of the synthesised nanoparticles for further analysis were as follow: $5.61 \times 10^{-10} \pm 3.37 \times 10^{-14}$ M for MIN and $6.50 \times 10^{-7} \pm 1.95 \times 10^{-10}$ M for NIN.

With the goal of creating a Point of Care (PoC) device, the selected nanoparticles were implemented on gold interdigitated electrodes (IDE) having an interdigital spacing of 5 µm. To analyse the sensors performance, Faradaic and non-Faradaic Electrochemical Impedance measurements were conducted. It was found that fabricated sensors were able to determine the SARS-CoV-2 spike protein showing a sensitive and reproducible response within different batches of immobilised electrodes. To prove its viability as a PoC sensor, spike protein concentrations were measured in biological fluids.

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P32. DEVELOPMENT OF AN AF4-MALS-ICP-MS/MS PLATFORM FOR ADVANCED EVALUATION AND QUANTIFICATION OF PROTEIN CORONA

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Keywords: *protein corona, AF4, ICP-MS/MS*

One current trend in bioanalysis is focused on the development of highly sensitive (nano)analytical tools based on signal amplification schemes and/or novel improved bioassays and biosensors. In this context, the integration of nanotechnology with analytical chemistry has received increasing attention over the last two decades, leading to the application of engineered nanomaterials to many different bioanalytical problems. In this sense, understanding the interaction between nanoscale technologies and the biological environment, the so-called nano-bio interface, is critical for the successful and safe application of nanomaterials in the life sciences. It is worth mentioning here that once the nanoparticles (NPs) are transferred to a biological medium, a superstructure called the protein corona is potentially formed. A shell of naturally present biomolecules, mainly proteins but also other biomolecules such as lipids and sugars, can surround the nanoassembly. Interestingly, the protein corona could significantly alter the physicochemical properties of the nanoparticles. Understanding the formation and nature of this corona is essential, especially for biomedical applications, as it influences NP behaviour, biodistribution, and efficiency of their applicañtion [1]

This study presents a novel method based on AF4-MALS-ICP-MS/MS for the quantification of the corona protein that could be formed when inorganic nanoparticles (e.g., AuNPs, CDs, QDs, etc.) are exposed to an environment rich in biomolecules. The approach combines asymmetrical flow field-flow fractionation (AF4) with multi-angle light scattering (MALS) and inductively coupled plasma mass spectrometry (ICP-MS/MS) to accurately analyze and measure the protein's presence and characteristics. This integrated technique offers high sensitivity, specificity, and precision, making it a valuable tool for studying corona proteins in complex biological samples. [2]

The method's effectiveness is demonstrated through experimental validation, highlighting its potential applications in biomedical research and nanotechnology.

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P33. DIRECT QUANTIFICATION OF INTACT PHOSPHOPROTEINS USING MIXED-MODE CHROMATOGRAPHY MS

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Keywords: *Phosphorylation, ICP-MS/MS, Mixed-mode HPLC*

Phosphorylation is one of the most important post-translational modifications (PTMs), which control signal transduction, enzyme activity, and protein-protein interactions in all living systems. However, the selective analysis of intact phosphoproteins is still a challenge due to low stoichiometry, structural heterogeneity, and matrix interferences. Traditional approaches such as antibody-based assays (e.g., western blot, ELISA), peptide-level mass spectrometry after digestion and enrichment (e.g., IMAC, TiO_2), phospho-specific staining, or radioactive labeling often require extensive sample preparation, introduce biases, or provide only peptide-level information and limited ability to quantify intact proteins and determine their global phosphorylation degree.

In this study, we introduce a method for absolute quantification and identification of intact phosphoproteins employing a single mixed-mode column that combines reversed-phase and anion-exchange stationary phases. Phosphorylated proteins exhibit enhanced retention compared to non-phosphorylated proteins due to their phospho- groups. On one hand coupling to triple-quadrupole ICP-MS/MS detection allows direct, element-specific detection of sulfur (S) and phosphorus (P)[1][2]. This configuration enables absolute quantification of phosphate stoichiometry on intact proteins without digestion, labeling, or isotopic standards. On the other hand, capLC-ESI-MS/MS allows the identification of phosphorylated proteins as in a single analysis we can separate non-phosphorylated proteins from phosphorylated proteins. This means that there is no ionization suppression from non-phosphorylated proteins as they do not coelute.

Compared to existing phosphoprotein assays, our approach provides higher selectivity, eliminates interference from non-phosphorylated proteins, and delivers robust, sequence-independent quantification. This strategy enhances reproducibility, simplifies workflows, and offers a reliable tool for profiling phosphoproteins in complex biological samples.

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P34. PENTAMETHINECYANINES AS BIOCIDES IN GRAM-POSITIVE AND GRAM-NEGATIVE BACTERIA

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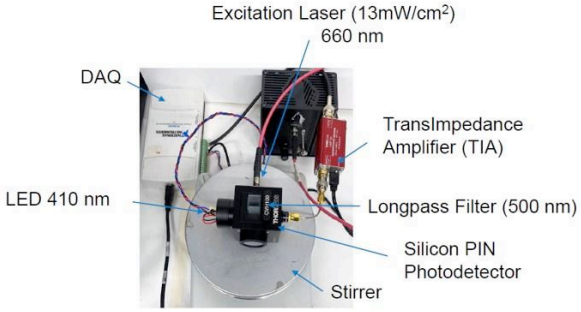
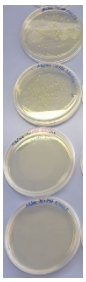
Keywords: *pentamethine cyanines, photodynamic therapy, bactericide*

Concern about antibiotic resistance has promoted the exploration of innovative treatments to inhibit pathogenic microorganisms. This study focuses on the synthesis, spectroscopic characterization, and potential application of two pentamethine cyanines, SP24 and NP11, as biocidal agents for antimicrobial photodynamic therapy (PDT)[1]. This technique employs photosensitizers activated by Near-Infrared (NIR) light to generate singlet oxygen and other highly oxidative species capable of damaging surrounding species.

Both dyes have been characterized by UV-Vis-NIR spectrophotometry and fluorescence. Additionally, a customized experimental setup has been developed to determine the singlet oxygen generation quantum yield, using methylene blue as a reference. This optical system uses two different light sources, one to excite the compound and generate the singlet oxygen and the other to measure the oxygen generation (Figure 1 A) with the marker 1,3-diphenylisobenzofuran (DPBF).[2] This equipment is employed for the antibacterial treatment in solution as well. The antimicrobial efficacy of the synthesized dyes has been tested on four bacterial strains: *Escherichia coli* and *Pseudomonas aeruginosa* (gram-negative), as well as *Staphylococcus aureus* and *Micrococcus luteus* (gram-positive).

Results show that SP24 and NP11 act as effective photosensitizers (Figure 1 B), exhibiting significant bactericidal activity when exposed to NIR light. Their mechanism of action is linked to their ability to generate singlet oxygen and interact with bacterial membranes, particularly in gram-positive bacteria, which are more vulnerable to hydrophobic interactions.





1. Negative control in PBS
2. Dark control with NP11
3. Treatment NP11/ 15 min radiation
4. Treatment NP11/ 30 min radiation

Figure 1. A) Customized and miniaturized optical set-up for the measurement of singlet oxygen and PDT of bacteria solutions. B) *M. luteus* growth studies using NP11 as photosensitizer.

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P35. EXOSOMES AS A NOVEL PHOTOSENSITIZER DELIVERY SYSTEM FOR ENHANCED PHOTODYNAMIC THERAPY FOR BREAST CANCER

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Keywords: *Photodynamic therapy, human milk exosomes, drug delivery system, breast cancer.*

Photodynamic therapy (PDT) is a non-invasive and promising alternative approach for cancer treatment that employs light irradiation in combination with photosensitizer (PS) to generate cytotoxic reactive oxygen species (ROS), leading to selective destruction of malignant cells. The success of PDT largely depends on two key factors: the selective accumulation of PS in tumor cells and the precise localization of light irradiation, both of which are essential to minimize damage to healthy tissues. However, several limitations hinder the broader application of PDT, including poor solubility, aggregation of PS, low biocompatibility, and inefficient tumor targeting [1]. Therefore, the development of safe and effective delivery systems for PS is critical to advancing the efficacy of PDT.

In recent years, exosomes have emerged as promising drug delivery vehicles for cancer therapy. Exosomes are nanoscale extracellular vesicles (30–150 nm) secreted by most cells, enclosed by a lipid bilayer and carrying a range of biologically active molecules, including proteins, lipids, carbohydrates, and nucleic acids.

Using exosomes as delivery system for PS offers numerous advantages over conventional drug carriers. Specially, exosomes exhibit high biocompatibility and low immunogenicity, reducing the risk of adverse immune responses. Additionally, exosomes show remarkable stability and the ability to traverse biological barriers, selectively targeting specific cells or tissues. This targeted delivery enhances therapeutic efficiency while minimizing side effects on healthy tissues. Moreover, exosomes protect their cargo from enzymatic degradation, further enhancing the stability and efficacy of the encapsulated drugs [2].

The aim of this study was to investigate the potential of human milk-derived exosomes as a novel carrier for the photosensitizer Fotoenticine in the photodynamic therapy of breast cancer. Fotoenticine was loaded into milk-derived exosomes using both passive (incubation) and active (freeze–thaw and sonication). The resulting drug-loaded milk-derived exosomes were characterized using various analytical methods to assess particle size, morphology, and drug loading efficiency. I*n vitro* studies using a breast cancer cell line demonstrated that Fotoenticine-loaded exosomes were significantly more effective in photodynamic therapy compared to free (non-encapsulated). This study highlighting the potential of exosomes as efficient nanocarriers for drug delivery in cancer therapy.

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P36. PROVIDING NEW INSIGHTS INTO SINGLE-PARTICLE ICP-MASS SPECTROMETRY

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Keywords: Single-particle ICP-MS, micro/nano-particle characterization, calibration strategies, data treatment

In recent years, the widespread use of nanomaterials has led to an increasing demand for analytical techniques capable of detecting and characterizing micro/nano-particles across a wide variety of applications. In this context, single-particle inductively coupled plasma mass spectrometry (SP-ICP-MS) has emerged as a powerful and versatile tool, attracting increasing attention from the scientific community.[1] Compared to conventional particle analysis techniques, SP-ICP-MS offers the unique advantage of determining both the particle size and number concentration in a single measurement. Thanks to this capability, the technique has gradually found application in a variety of areas, ranging from materials science to health-related and technological fields.

In SP mode, the ICP-MS unit operates at high data acquisition frequencies to detect discrete entities such as nanoparticles. By analyzing sufficiently diluted suspensions, only one particle at a time is introduced into the plasma, generating an ion cloud that produces a sharp, transient signal upon reaching the detector. The intensity of each peak is proportional to the analyte mass within the particle and, following appropriate calibration, enables quantification. If the chemical composition and shape of the particles are known or assumed, the mass can be used to calculate particle sizes, from which the particle size distribution can be derived. At the same time, the number of detected events during the acquisition period provides information on the particle number concentration.[2]

Although the technique is progressively consolidating its foundations and moving closer to routine application in various fields, there is still significant room for further investigation into its underlying principles and methodological optimizations. Building on the current state of SP-ICP-MS, this work proposes new calibration strategies and data processing approaches aiming at enhancing the technique's performance. In particular, the focus is on expanding the scope of extractable information, thus facilitating particle characterization tailored to the specific objectives of each study. As a proof-of-concept, the methods were applied to real-world samples relevant to materials science and valuable environmental samples to assess their micro/nano-particulate content.

Acknowledgements

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P37. IN VITRO AND *IN VIVO* APPROACHES FOR THE ASSESSMENT OF THE ANTITUMORAL CAPACITY OF POMEGRANATE SEED EXTRACTS

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Keywords: *Anticancer, proteins, peptides, apoptosis*

The global rise in pomegranate consumption, driven by its numerous health benefits, has led to a substantial increase in associated waste. Among this waste, pomegranate seeds represent an affordable and eco-friendly source of protein that remains largely underexplored. Previous studies carried out by our group demonstrated the antioxidant potential of protein- and peptide-enriched extracts derived from pomegranate seeds, obtained through green extraction techniques: high-intensity focused ultrasound (HIFU) and pressurized liquid extraction (PLE) [1]. Building on this background, the current study explores the antitumor effects of these protein and peptide isolates from pomegranate seeds obtained using HIFU and PLE. A comprehensive range of cancer-associated cellular processes was examined in two cancer cell lines: HCC1806 for breast cancer and PC-3 for prostate cancer. The processes studied included viability, migration, clonogenicity, adhesion, cell cycle progression, and metalloproteinase (MMP) activity. To validate the *in vitro* findings, *in vivo* studies were conducted using immunodeficient mice (Hsd:Athymic Nude-Foxn1nu). Tumor weight and volume, along with vascular endothelial growth factor (VEGF) levels and MMPs activity, were evaluated in treated mice.

Results showed that all tested extracts significantly reduced cell viability within the concentration range of 0.1 to 15 mg/mL, with lower IC_{50} values observed in breast cancer cells compared to PC-3 cells. Based on these results, specific concentrations were selected for further analysis: 4 mg/mL for PLE protein isolates, 2 mg/mL for HIFU protein isolates, and 2.4 mg/mL for hydrolysates. Whereas all treatments markedly inhibited cell migration and proliferation in both cancer cell lines, their effects were different on MMP activity, cell adhesion, and cell cycle. In HCC1806 cells, proMMP-9 and MMP9 activity was reduced, but Pro-MMP2 remained unaffected. In contrast, in PC-3 cells, all extracts suppressed MMP9 activity, and hydrolysates further reduced Pro-MMP9 and Pro-MMP2 activity. Regarding adhesion, it was enhanced in HCC1806 cells with all treatments, while in PC-3 cells, this effect was only observed with the HIFU-derived hydrolysate. Finally, cell cycle analyses showed S-phase arrest in response to HIFU extracts and apoptosis induction by PLE extracts in both cell lines. *In vivo* studies corroborated these effects, showing significant reductions in tumor volume and weight following treatment with the hydrolysates. VEGF levels and the activity of MMP-2 and MMP-9 (both active and pro-forms) were also altered in treated tumors. Overall, these findings support the potential of pomegranate seed-derived compounds as antitumor agents.

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P38. ULTRASENSITIVE DETECTION AND QUANTIFICATION OF OXYGENATED COMPOUNDS IN COMPLEX SAMPLES USING GC-COMBUSTION-MS

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Keywords: *GC, MS, Oxygen.*

We present a novel and highly selective Gas Chromatography (GC) detection method designed for the accurate quantification of volatile oxygen-containing compounds at ultratrace levels in complex matrices, without the need for compound-specific standards. The approach is based on the use of isotopically enriched oxygen-18 as the oxidizing gas in a combustion oven operated at 800 °C.This method enables the detection of the natural oxygen present in the individual GC-separated compounds by incorporating it into the volatile species formed after combustion and subsequent degradation to ^{16}O (m/z 16) in the ion source. The unspecific signal from the ^{16}O abundance on the oxidizing gas (and the residual air contamination) was compensated by m/z 12 signal originating from CO_2 degradation in the ion source. The method achieved a detection limit of 28 pg of oxygen injected, the lowest reported for any GC detector. Even in challenging cases where oxygenates co-eluted partially or completely with major matrix components, the detection limit only increased to 55 and 214 pg, respectively. The system also exhibited excellent equimolar response, linearity, and peak shape.

Validation was conducted by analyzing Standard Reference Material (SRM), producing results that were accurate (99-103%) and precise (< 4% RSD). Robustness was tested by spiking a hydrotreated diesel with ten oxygen-containing compounds at the low ppm levels. Despite the sample's complexity, each oxygen compound could be discriminated from the matrix and accurately quantified, with a mean recovery of 102 ± 9%. Furthermore, the method was successfully applied to determine the concentration of different oxygenates naturally present in a wood biooil sample.

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P39. MAGNETIC MOLECULARLY IMPRINTED POLYMER FOR SELECTIVE EXTRACTION OF PATULIN FROM APPLE JUICES USING ULTRA-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY COMBINED WITH MASS SPECTROMETRY

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Keywords: *patulin, mycotoxin, magnetic molecularly imprinted polymer, high-resolution mass spectrometry, apple juice.*

A simple, rapid, and sensitive method for the determination of patulin in apple juice has been developed using dispersive magnetic solid-phase microextraction (DMSPE) with synthetized ferrite magnetic molecularly imprinted polymers (MMIPs) followed by ultra-high performance liquid chromatography-high resolution mass spectrometry (UHPLC-HRMS). Patulin mycotoxin can be found in fruits, generally apple[1], and its consumption results in the most recognizable source of human exposure to this toxin. Therefore, highly selective extraction techniques are needed to determine patulin in apple juice and improve food safety, where MMIPs may offer considerable opportunities given their high specificity and the complexity of plant-based matrices. Additionally, the method enables high preconcentration levels, and the magnetic properties of MMIPs simplify sample preparation.

Sample treatment was optimized using a multivariate experimental design for adsorption parameters including the mass of MMIPs, salt concentration and adsorption time. The desorption solvent and its volume and the desorption time were the optimized parameters for the desorption step of the patulin from the MMIP material. A final mass of 45 mg of MMIP added to 1 mL of juice was submitted to adsorption and desorption optimized steps before UHPLC-HRMS analysis. Under optimized conditions, the developed method demonstrated excellent linearity over a concentration range of 5 to 100 ng mL^{-1}, with a correlation coefficient of 0.989 and a limit of quantification of 1.09 ng mL^{-1}.

Accuracy was confirmed through recovery studies, achieving values between 96% and 106%. The analytical method was applied to commercially available apple juices. A total of 10 juice samples were analyzed, and patulin was detected in one of the samples at 4.8 ± 0.4 ng mL^{-1} concentration. In addition, an untargeted approach was performed and 2 patulin degradation products were detected in 3 samples. Additionally, the elemental composition, morphology and a study on the reusability of the MMIP were assessed.

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P40. PHOTOLYTIC PROCESSES FOR THE DEGRADATION OF TRIAZOLES: IDENTIFICATION OF TRANSFORMATION PRODUCTS BY MICROEXTRACTION AND LIQUID CHROMATOGRAPHY COUPLED TO HIGH-RESOLUTION MASS SPECTROMETRY

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Keywords: *fungicides, transformation products, dispersive liquid-liquid microextraction, screening strategies*

Triazole derivatives, such as difenoconazole (DFC), tebuconazole (TBC) and triadimenol (TDM), are fungicides widely used in agriculture that can persist in surface and groundwater and are highly toxic to aquatic ecosystems and human health.[1] Photolytic degradation processes are usually addressed to water remediation, with or without the presence of catalysts to enhance pollutant degradation. However, these degradation processes may result in the generation of transformation products (TPs) with unknown environmental risks.[1] As TPs can be generated at trace levels, very sensitive analytical methods are required for their identification. Thus, well-established microextraction techniques can be applied to enhance method sensitivity. Dispersive liquid-liquid microextraction (DLLME) has demonstrated its effectiveness in extracting organic compounds of non-polar nature, as well as its simplicity, rapidity and environmental friendliness.[2]

This work focuses on the monitoring of DFC, TBC, TDM and their corresponding TPs during degradation experiments. Degradation trials were carried out in a photoreactor applying ultraviolet A irradiation, with light emitting diode lamps, on milli-Q water containing 10 mg L^{-1} of DFC, TBC and TDM, with or without the presence of TiO_2 and $Na_2S_2O_8$ as catalyst and co-oxidant, respectively. The extraction of the triazole compounds and their TPs was carried out using DLLME and the enriched extracts analyzed by liquid chromatography coupled to high resolution mass spectrometry with quadrupole time-of-flight (LC-QTOF) analyzer for identification purposes. LC-QTOF operated in AutoMS/MS mode to carry out suspect and non-targeted screenings. Home-made databases with 31 DFC TPs, 129 TBC TPs and 25 TDM TPs described in literature were made for suspect screening. Non-targeted screening, without considering any database, consisted of the spectral investigation of a deconvoluted and aligned list of peaks with suitable degradation trends. Finally, 18 DFC TPs, 19 TBC TPs and 9 TDM TPs were found through both strategies. On the other hand, LC combined with tandem MS (LC-MS/MS) with triple quadrupole analyzer was used for quantification purposes.

The proposed methodology was applied to wastewater treatment plant (WWTP) samples, fortified with 0.2 mg L^{-1} of DFC, TBC and TDM, and exposed to natural sunlight during March 2025 in Murcia (Southeast of Spain) in the presence of TiO_2 and $Na_2S_2O_8$.

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P41. COPPER INK-BASED NANOCRYSTAL SUBSTRATE FOR ENHANCED RAMAN SPECTROSCOPY

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Keywords: *Copper ink, point-of-care analysis, SERS, nanomaterials*

Raman spectroscopy is an analytical technique that offers insights into molecular structure and chemical composition. This non-destructive method employs inelastic light scattering to identify chemical compounds with high specificity. Its applications extend across chemistry, biology, materials science, and pharmaceuticals, enabling the analysis of solid, liquid, or gas samples with minimal preparation. The technique's capability to detect molecular changes makes it invaluable for manufacturing quality control, forensic analysis, and environmental monitoring. Recent advancements in Surface-Enhanced Raman Spectroscopy (SERS) have improved detection limits for trace analysis and biomedical applications.

In this work, we introduce an innovative and versatile methodology for detecting various analytes, supported by the *in-situ* generation of a substrate (nanocrystal) that enhances the Raman signal. In previous studies performed by our group, the Raman substrate responsible for the enhancement of the Raman signal was electrochemically synthesized during the reduction or oxidation of the electrode. However, in the present study, the substrate was chemically generated due to the reactivity of copper serigraphy inks under specific conditions.

Nanocrystals are formed by chemical oxidation of a copper ink, generating various structures such as CuCl or CuI. The chemical attack is performed in the presence of anions (Cl^-, I^-, Br^-), which strongly adsorb on the nanocrystal surface, resulting in a negatively charged surface. This surface charge eases the adsorption of several analytes, allowing its SERS detection. By varying the medium's conditions, such as acid concentration, we were able to elicit a better response from the charged nanocrystal, leading to a Raman increase by several orders of magnitude.[1] This methodology allows a sensitive detection of a variety of analytes within seconds using inexpensive materials.

This study shows a novel approach for the *in-situ* generation of SERS-active substrates by chemical attack of highly reactive copper inks. This technique shows promise for various analytical applications, especially for the development of point of care applications, where fast and user-friendly analysis are critical. Further research is warranted to explore the full potential of this method across different analytes and to optimize the process for practical implementations.

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P42. PREPARATION OF CHITIN-CHITOSAN-PECTIN NANOPARTICLES TO ENHANCE ASTAXANTHIN SOLUBILITY VIA ULTRASONICATION

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Keywords: *biopolymers, nanoparticle, ultrasound, functional food, astaxanthin*

Astaxanthin (ASTX) is a carotenoid pigment recognized as one of the most potent natural antioxidants. However, its lipophilic nature and highly unsaturated chemical structure make it susceptible to degradation under both storage conditions and physiological conditions [1]. To overcome these limitations, encapsulation systems have emerged as effective strategies to protect ASTX from environmental stressors, while also promoting sustained release and bioavailability in the gastrointestinal tract.

Food-grade biopolymers are particularly attractive for ASTX encapsulation due to their safety, biodegradability, low cost, and ability to stabilize bioactive compounds. To attain suitable candidates for nanoparticle (NP) formulation, various natural origin biopolymers-including amylopectin, chitin, and pectin, as well as chitin-tween 80 combination, were initially evaluated. Based on key performance criteria, a partially deacetylated chitin-chitosan mixture (CH/CHS) combined with pectin (PE) was selected for further study, owing to its favorable characteristics such as reduced nanometric size, low polydispersity index (PdI), high zeta potential value, and superior encapsulation efficiency (EE %) [2,3].

Nanoparticles were prepared using ultrasonication, leveraging the electrostatic interaction between protonated amino groups of the CH/CHS mixture in acidic conditions and negatively charged pectin. This method enabled the formation of a stable polyelectrolyte complex without the need for chemical crosslinking agents [4].

The effects of physic-chemical and instrumental variables related to key formulation, namely, amplitude, sonication time, CH/PE ratio, and ASTX concentration, were evaluated on ensuing meaningful nanoparticle responses: nanoparticle size, PdI, zeta potential, and EE % by means of a four-factor, three-level Box-Behnken Design (BBD) Response Surface Methodology (RSM).

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P43. ALL-IN-ONE SUPRAMOLECULAR SOLVENT-BASED EXTRACTION FOR THE MULTICLASS DETERMINATION OF EU-REGULATED MYCOTOXINS IN FOODSTUFFS

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Keywords: *Supramolecular solvents; mycotoxins; food regulation; food analysis; microextraction; liquid chromatography-mass spectrometry*

Mycotoxins are among the top three most frequently reported hazards in the Rapid Alert System for Food and Feed (RASFF), affecting a wide range of food products within and outside the European Union. Due to their recognized carcinogenicity, classified by the International Agency for Research on Cancer (IARC) in Groups I, IIA, and IIB, regulatory limits have been set for their presence in various food matrices. Thus, it is necessary the development of analytical methods able to determine multiple mycotoxins simultaneously at legally set maximum concentrations.

Traditional extraction techniques based on petroleum-derived solvents, such as QuEChERS, solid-phase extraction, and immunoaffinity columns, are widely used. However, they often require matrix-specific protocols and multiple clean-up steps, increasing solvent consumption, complexity, and costs. Furthermore, combined extraction method is required for the determination of mycotoxins with different physicochemical properties.

Supramolecular solvents (SUPRAS) provide an innovative “all-in-one" extraction strategy. Their nanostructured composition and multiple interaction mechanisms enable efficient solubilization of analytes across a broad polarity range, while reducing matrix interferences. SUPRAS allow multiextraction and clean-up in a single, rapid step, supporting high-throughput workflows.

In this study, a SUPRAS-based microextraction method coupled with liquid chromatography–tandem mass spectrometry (LC-MS/MS) was developed for the simultaneous determination of twelve EU-regulated mycotoxins. The method was optimized and validated across five categories of solid and liquid food matrices, following the performance requirements of Regulation (EU) No. 519/2014. The procedure showed excellent sensitivity (limits of quantification ranged from 0.07 to 25.48 ng/mL), suitable extraction recoveries (69–115%), and high precision (relative standard deviations of 0.3–20% for repeatability and 3–19% for reproducibility).

All-in-one SUPRAS extraction offers a simple, rapid, and sustainable solution for mycotoxin determination, representing a major advance towards greener, faster, and more cost-effective food safety monitoring.

Acknowledgements

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P44. ARCHAEOMICS: STRATEGY TO IDENTIFY SPECIFIC FECAL BIOMARKES OF ANIMALS IN ANCIENT SAMPLES

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Keywords: *fecal biomarkers, Archaeomics, ancient samples, HRMS*

The unequivocal identification of the animal species housed throughout the domestication is a crucial point for the archaeologists to describe the development of the domestication. Different strategies can be used for this purpose such as micromorphological, archaeobotanical, zooarchaeological, and/or chemical studies. According to the chemical studies, commonly, proxies of faecal biomarkers concentrations (sterols, phytosterols and bile acids) are used to determine the animal housed[1]. Nevertheless, these proxies use ratios obtained from contemporary samples concentrations which are applied to ancient samples considering the same degradation rate for all the compounds. Besides, any of the faecal biomarkers is specific so when different species were housed in the same site, the identification is quite difficult. Consequently, the search of specific and persistent faecal biomarkers is necessary. In this sense, a new term can be defined, Archaeomics, the search of specific and persistent biomarkers which describes ancient activities. Therefore, the aim of this study is to describe the strategy carried out to obtain specific and persistent biomarkers to identify the animal species housed in ancient sites.

Sampling: Faecal matter of different animal species (goat, sheep, horse, cow, suids) and location were collected. The feeding of the animals was natural, and the faecal matter was collected before being parasitised.

Homogenization: All the samples were freeze and dried, milled and sieved.

Sample treatment: 0.1 g were extracted with 5 mL methyl-*tert*-butyl ether and then, 5 mL of water were added to eliminate extracted polar compounds. The organic layer was collected and evaporated to dryness. Finally, 2 mL of methanol were added to dissolve all the analytes.

Analysis: All the analysis were carried out in a LC-qTOF analyser. All the chromatographic and analysis parameters were previously optimized, and blanks and QC were also analysed.

Data treatment: Features were obtained from Profinder software at positive and negative ionization modes. Principal Component Analysis was performed with raw data toa check the grouping of the samples. sPLS-DA and PLS-DA models were created. Features discrimination was done using Variable Importance in Prediction (VIP) score values. Specific features for each animal species were obtained except for cow and sheep. A new model was done with just those selected features and 100% of effectiveness was obtained.

Persistence of the features: Previously analysed and classified ancient sediment samples from El Mirador Cave (Sierra de Atapuerca, Burgos) and Vallone Inferno rock-shelter (Sicily, Italy) were used determine the persistence of the features. The same treatment and analysis was carried out with these samples and previously selected features were identified.

Selection of the features: Just the selected features detected in the ancient sediment samples were used for their identification. Just goat and suid specific features were persistent.

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P45. DETERMINATION OF STEROID PHASE II METABOLITES: AN ANALYTICAL CHALLENGE TO PROVIDE NEW INSIGHTS IN HORMONAL CHANGES DURING PREGNANCY

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Keywords: *Steroids; Metabolomics; Pregnancy; LC-MS/MS; Bioanalysis.*

Steroid hormones play a critical role in several physiological processes such as pregnancy. Thus, the determination of steroid hormones and metabolites, aka steroid profile, is a valuable tool to evaluate the variation of these hormones during pregnancy and their impact in health [1]. The common analytical strategy for the determination of the steroid profile is based on the hydrolysis of the phase II conjugates, the extraction (either LLE or SPE) of the released steroids and their determination by GC-MS after derivatization. The occurrence of LC-MS/MS and its routine implementation in most laboratories opened the door for the direct detection of phase II metabolites. This direct detection not only simplifies the analytical approaches used for the detection of the steroid profile but also expands the knowledge about the steroidome since specific information on the balance between the different conjugates can be obtained.

However, direct determination of phase II steroid metabolites presents several important analytical challenges which are delaying its common implementation in routine steroid analysis. Thus, analytical methods aiming to directly determine phase II steroid metabolites should face up to (i) the wide number of species formed e.g. mono-sulfates, glucuronides, sulfo-glucuronides, bis-sulfates, bis-glucuronides, (ii) the high polarity of some metabolites such as bis-sulfates, (iii) the large number of isomeric species and (iv) the structural similarity of the steroid metabolites [2].

In this presentation, we describe the analytical considerations for the proper determination of these challenging metabolites. We also present the application of the optimized conditions to evaluate the hormonal changes during pregnancy and their association with brain structure [3] and postpartum depressive mood [4].

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P46. METABOLIC PROFILING AND FUNCTIONAL PATHWAY ANALYSIS IN THE DIGESTIVE GLAND OF FRESHWATER MUSSELS (*UNIO RAVOISIERI):* IMPACT OF NANOPARTICLE EXPOSURE, AND THE PROTECTIVE ROLE OF SELENIUM

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Keywords: *Unio Ravoisieri, ZnTi Nanoparticles, Selenium, Environmental Metabomolics*

Nanoparticles (NPs) are widely used in cosmetics, pharmaceuticals, and water treatment due to their due to their biocompatibility and antimicrobial properties [1,2]. However, their increasing presence in aquatic ecosystems raises concerns regarding potential ecotoxicological effects on aquatic organisms [3,4].

This study aimed to evaluate the metabolic disturbances and functional pathway alterations in the digestive gland of the freshwater mussel *Unio ravoisieri* after exposure to Zinc-Titanium nanoparticles (ZnTiNPs). Additionally, it assesses selenium' (Se) protective role in restoring metabolic homeostasis against nanoparticle-induced toxicity.

Mussels were exposed to two concentrations of ZnTiNPs (10 and 100 µg/L), either alone or in combination with 100 µg/L of Se, over 14 days. Untargeted metabolomic analysis of digestive glands was performed using complementary analytical platforms: GC-MS and UHPLC-QTOF-MS in both electrospray ionization positive (ESI+) and negative (ESI−) modes offering extensive metabolic coverage.

ZnTiNPs exposure significantly altered the mussels' metabolome, impacting fatty acid biosynthesis, amino acid pathways, and key metabolic routes, including glutathione, glyoxylate, and dicarboxylate metabolism. Se supplementation counteracted several of these metabolic disruptions, suggesting its potential protective role against nanoparticle-induced toxicity.

The combination of GC-MS and UHPLC-QTOF-MS provided comprehensive insights into the metabolic and functional pathway alterations caused by ZnTiNP exposure. Se supplementation effectively modulated these disruptions, demonstrating its potential as a key mitigator of nanoparticle-induced toxicity. These findings highlight Se role in restoring metabolic homeostasis, underscoring its significant protective effect against environmental nanotoxicity in aquatic organisms.

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P47. COUPLING OF MAGNETIC SOLID PHASE EXTRACTION WITH CAPILLARY ELECTROPHORESIS FOR THE AUTOMATIC DETERMINATION OF COPPER IN WATER

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Keywords: *Copper, automatic determination, magnetic solid phase extraction, capillary electrophoresis*

In this work, a reliable and rapid capillary electrophoresis (CE) method for the determination of Cu in water samples was developed. First, a chelant nanomaterial based on magnetic nanoparticles (MNPs) functionalized with (3-Aminopropyl)triethoxysilane (MNPs-APTES) was synthesized and evaluated as a sorbent towards several inorganic cations. Then, MNPs-APTES was applied to develop an automated magnetic solid-phase extraction method (MSPE) coupled with CE (MSPE-CE) for the determination of Cu in water samples. To achieve this, the preconcentration and the determination of the target analyte were performed in a single run, separating the analyte from potential interferents. The extraction of Cu and its determination in the MSPE-CE system was achieved with a simple electrolyte composed of imidazole and acetic acid [1]. The effects of the amount of functionalized MNPs, sample volume, extraction pH, and eluent composition on the extraction efficiency were systematically studied. The developed method, under optimal conditions and combined with diode array detection, showed high sensitivity, an extended linear range, and low sample consumption, presenting spiked recoveries in real samples close to 100 %. Sample preparation consisted of dilution, and filtration. To the best of our knowledge, this is the first report of an automatic MSPE-CE system for Cu determination.

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P48. VERTICAL FLOW IMMUNOELECTROANALYTICAL ASSAY FOR DECENTRALIZED AND DIFFERENTIAL DIAGNOSIS OF STROKE

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Keywords: *Decentralized analysis, Differential stroke diagnosis, Vertical flow immunoassay, Immunoelectroanalysis*

Biochemical analysis is continuously evolving and new methodologies for obtaining **fast and accurate information** are required to make **relevant decisions**. This is the case of the **differentiation between ischemic and hemorrhagic strokes in acute phase**, where the decisions taken have consequences not only on the survival but also in the quality of life of people having suffered a stroke episode.

Nowadays, diagnostic techniques rely on neuroimaging techniques, which are not always readily available, especially in outpatient facilities. Biochemical analysis of stablished biomarkers is commonly performed in serum samples, or alternatively, in cerebrospinal fluid, obtained after a painful and highly invasive lumbar punction. Thus, the use of **minimally invasive samples** as a source of biomarkers, along with **simple but accurate methodologies** is required for moving the analysis to where it is required.

Flow immunochromatographic assays, especially lateral flow immunoassays, have demonstrated to be a fast method to obtain qualitative or semiquantitative information. Strategies are being designed and evaluated to integrate quantitative detection into the platforms, but this is not an easy task. **Electrochemical detection**, due to its intrinsic possibilities of miniaturization, fits perfectly for decentralized approaches[1,2]. Moreover, in the less explored **vertical flow format**, the design could employ an electrochemical card as the backing pad where the different functional and structural layers are overlayed. Thus, the sample would flow directly to the detection system, that in this case is part of the design.

A vertical flow immunoelectroanalytical assay is here shown for the determination of **glial fibrillary acidic protein (GFAP)**, a biomarker that has demonstrated its potential for the differential diagnosis of stroke in serum, and nasal exudate[3]. A compact and robust design is here presented, together with the milestones that are required for real implementation.

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P49. CHIRAL ANALYSIS OF THYROID HORMONES IN HUMAN MILK: POTENTIAL ASSOCIATIONS WITH MICROBIOTA

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Keywords: *Thyroid hormones, human milk, column.switching, chirality, microbiota, speciation, chromathography, hollow fiber*

Iodine is an essential micronutrient that can only be obtained through diet, with increased requirements during pregnancy to support maternal thyroid adaptations and fetal development. Previous studies have investigated the impact of iodine deficiency on the human milk (HM) metabolome[1]. Thyroid hormones (TH) are the sole iodine-containing bioactive compounds, playing a crucial role in infant health. These hormones possess chiral centers, and their enantiomers exhibit distinct metabolic and biological efficiencies. While D-thyroxine (D-T4) does not significantly impact metabolic rate, L-thyroxine (L-T4) actively regulates it. However, D-T4 has been shown to influence hepatic lipogenic enzyme levels, reduce plasma cholesterol, and suppress thyrotropin (TSH) secretion. Despite the well-documented higher efficiency of L-T4 compared to its D counterpart [2], the presence of chiral TH in HM has not been previously reported.

In this study, microbiota profiling of HM was performed through 16S rRNA gene amplicon sequencing on the Illumina platform. Our findings revealed a distinct microbiota composition differentiating iodine-deficient mothers from control groups. Additionally, we quantified TH in HM to find potential associations between microbiota and TH in HM. To this end, eight TH were determined in HM using a column-switching system integrated into an ultra-high performance liquid chromatography (UHPLC) setup that combines chiral and reversed-phase stationary phases. TH were identified and quantified through the combination of quadrupole time-of-flight (QTOF) tandem mass spectrometry in parallel with inductively coupled plasma triple quadrupole mass spectrometry (ICP-QQQ-MS), and ion mobility mass spectrometry (IMMS). Sample preparation involved three-phase hollow-fiber liquid-phase microextraction (HF-LPME), a reliable, cost-effective technique that minimizes cross-contamination due to its disposable fiber. This method enabled the accurate quantification of eight TH at endogenous levels in HM. A total of 134 HM samples were analyzed, revealing TH concentrations (ng/g) in the following order: D-T4 (135 ± 5) > L-T4 (94 ± 17) > 3,3',5'-triiodothyronine (rT3, 65 ± 13) > 3,3',5-triiodothyronine (T3, 52 ± 10) > 3,5-diiodothyronine (T2, 37 ± 6) > 3,5-diiodotyrosine (DIT, 16 ± 2) > thyronine (T0, 14 ± 3) > 3-iodotyrosine (MIT, 14 ± 6). This study highlights the need for accurate analytical methods to explore intricate biological challenges, ultimately contributing to better nutritional and therapeutic approaches for mothers and infants.

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P50. EVALUATION OF A GROUP OF PROTEASES TO PRODUCE BIOACTIVE HYDROLYSATES FROM MANDARIN PEELS PROTEIN EXTRACTS OBTAINED BY MICROWAVE-ASSISTED EXTRACTION

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Keywords: *microwave-assisted extraction, mandarin peels, proteins, proteases, bioactive peptides.*

Agro-industrial by-products, such as fruit peels, represent an underutilized yet valuable source of bioactive compounds with potential applications in food, nutraceutical, and pharmaceutical industries. Mandarin (*Citrus reticulata*) peels, typically discarded during juice production, are rich in proteins and other functional compounds that can be valorised through innovative processing technologies.[1] Green extraction techniques aim to reduce environmental impact by minimizing the use of toxic solvents, lowering energy consumption, and enhancing overall process efficiency. Among these emerging technologies, Microwave-Assisted Extraction (MAE) has proven to be an effective and eco-friendly method for recovering proteins from plant-based matrices. [2] However, it has never been applied to protein extraction from citrus peels.

This study aimed to evaluate the potential of mandarin peel proteins, extracted using MAE, to generate bioactive peptides through enzymatic hydrolysis. Firstly, a Box-Behnken experimental design was employed to determine the best conditions to extract proteins from citrus peels. Temperature, extraction time, and ethanol percentage were optimized by measuring as response variables the protein content (maximized) and the Maillard compound formation (minimized). Then, protein extracts obtained under optimal conditions (130ºC, 5 min, and 50% EtOH) were subjected to hydrolysis using different commercial proteases: Thermolysin and Vertera Educe (metaloproteases), Alcalase 2.4L, Vertera Essence W (subtilisin type proteases), Vertera Rise and Vertera Smooth (serine proteases), Vertera Flavour (exopeptidase), and Vertera Savoury (exo and endopeptidase). The hydrolysis conditions (enzyme concentration and time) were optimized by measuring the peptide content for each enzyme under all the conditions studied. The hydrolysates obtained under the optimal conditions selected for each enzyme were then characterized through the evaluation of different bioactivities (antioxidant, antimicrobial, and antihypertensive). Finally, peptides present in the hydrolysates presenting the highest bioactivities were identified by UHPLC-MS/MS.

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P51. ELECTROCHEMICAL SENSOR BASED ON SCREEN-PRINTED ELECTRODES FOR THE DETECTION OF ANTICANCER AGENT REVUMENIB IN NON-INVASIVE CLINICAL SAMPLES

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Keywords: *adsorptive stripping square wave voltammetry, screen-printed electrodes, carboxyl functionalized multi-walled carbon nanotubes, revumenib, human urine.*

The menin inhibitor revumenib has emerged as a promising targeted therapy for patients with acute myeloid leukemia (AML) who have shown resistance to conventional treatments [1]. Despite its therapeutic potential, concerns regarding its safety profile remain, highlighting the need for further investigations to establish optimal dosing regimens and administration schedules. In this context, the development of innovative bioanalytical strategies for therapeutic drug monitoring (TDM) in clinical samples represents a current challenge in the field of Analytical Chemistry.

Among many analytical approaches available, electroanalytical techniques have demonstrated significant advantages for the analysis of pharmaceutical and biological fluids, including low cost, operational simplicity, rapid analysis and portability. Currently, one of the most promising miniaturized platforms is the use of sensors based on screen-printed electrodes (SPEs) [2]. Specially, carboxylated multi-walled carbon nanotubes (MWCNTs-COOH) have gained considerable attention as effective electrode materials in electrochemical sensing, owing to their remarkable physical, chemical and electrical properties. These features contribute to enhanced sensitivity, specificity and the potential for real-time diagnosis in point-of-care applications. However, its applicability to complex biological fluids should be demonstrated.

The objective of this study is to develop a selective and sensitive biosensor platform based on SPEs modified with MWCNTs-COOH for the determination of revumenib in human urine at clinically relevant concentrations. Through a carefully optimization of key parameters to maximize the electrochemical response, the device was applied to these non-invasive samples using adsorptive stripping square wave voltammetry. A simple dilution in phosphate buffer solution (0.1 M, pH 10) enabled the detection, identification and quantification of the anticancer agent in these biofluids. The developed device exhibited a linear analytical response (50-1000 nM), with limits of detection and quantification of 16 nM and 48 nM, respectively. Also, recovery studies were in the range of 92-111% with the possibility to use the same device up to eight times (RSD ≤8%). To the best of our knowledge, this is the first report describing the electrochemical behavior of revumenib using this sensing approach. These findings open new possibilities for implementing TDM strategies, supporting a shift from the conventional "one-size-fits-all" model toward personalized medicine in AML patient management.

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P52. MERCURY SELENIDE NANOPARTICLES ANALYSIS BY SINGLE PARTICLE ICP-MS TO ACHIEVE A COMPLETE MERCURY SPECIATION STUDY IN FISH TISSUES

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Keywords: mercury speciation, mercury selenide nanoparticles, single particle inductively coupled plasma mass spectrometry

Mercury selenide nanoparticles (HgSeNPs) are considered key agents in the detoxification mechanisms of mercury (Hg) due to the protective role of Se in living organisms [1]. Their incorporation into classical Hg speciation studies, which often focus on the traditional chemical forms of Hg (methylmercury (MeHg) and inorganic mercury (iHg)), contributes to a more comprehensive understanding of the environmental fate of Hg and its impact on the health of living organisms. For this reason, this work tests the classical Hg speciation analysis extraction conditions established in previous research [2] to determine their quantitative applicability for HgSeNPs analysis. The goal is to develop a sample preparation method that allows the simultaneous extraction of these species. Gas chromatography coupled with atomic fluorescence detection via pyrolysis (GC-pyro-AFS) was used for MeHg and iHg determination, and single particle inductively coupled plasma mass spectrometry (SP-ICP-MS) for HgSeNPs determination.

In this study, microwave energy was explored as a faster, and not previously investigated strategy, for the extraction of HgSeNPs, in combination with tetramethylammonium hydroxide (TMAH) which is an alkaline and low-cost reagent is commonly employed for classical Hg speciation. The optimization and validation of the methodology were performed with commercial HgSe, whose nanoparticulated fraction was characterized in terms of size, concentration and composition employing various techniques: Scanning Electron Microscopy (SEM), Energy-dispersive X-ray spectroscopy (EDX), Dynamic Light Scattering (DLS) and SP-ICP-MS. Different microwave-assisted extraction programs were studied by changing the maximum irradiation temperature. Commercial HgSe was spiked on fish tissues with low total mercury levels (salmon and blue withing muscles) to explore the extraction in several scenarios. These tissues were selected due to their low total mercury levels ensuring the absence of this type of nanoparticles and verifying that no HgSeNPs were generated under the selected extraction conditions. Finally, the developed method was applied to different fish muscle tissues (swordfish, tuna and tucunaré) with previously studied MeHg and iHg contents. In these tissues, HgSeNPs were detected in all cases and characterized in terms of concentration and size using SP-ICP-MS yielding satisfactory results. This work is notable for reducing both the sample preparation time and the amount of sample required, thanks to its simultaneous and common extraction approach, leading to a comprehensive Hg speciation.

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P53. SUBCRITICAL WATER EXTRACTION AND MICROWAVE-ASSISTED EXTRACTION AS SUSTAINABLE APPROACHES FOR PROTEIN RECOVERY FROM ORANGE PEELS

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Keywords: *Subcritical Water extraction, Microwave Assisted Extraction, Bioactive peptides, Orange peel*

According to the United States Department of Agriculture, world orange production for the 2024/2025 season is forecast at 45.2 million tons [1]. A high percentage of this production is used for juice extraction, which generates a large amount of waste such as peel, seeds, and pulp. If these wastes are not properly managed, they can lead to adverse environmental effects. However, there is noteworthy potential to turn these wastes into valuable products sustainably, thanks to the wide range of compounds with interesting bioactive properties that they can contain [2]. In this context, a growing interest has emerged in recent years to develop green extraction strategies that enable obtaining value-added compounds from food wastes and support a circular economy.

Considering that the peel is the main residue of this citric fruit, and it is a significant source of bioactive compounds, this work aimed to develop sustainable strategies for the extraction of proteins from orange peels. Thus, two different strategies based on subcritical water extraction and microwave-assisted extraction were developed to extract proteins from the peels of two orange varieties. To obtain bioactive peptides, the extracts were subsequently hydrolyzed using Alcalase and Thermolysin enzymes, and different bioactivity assays were carried out to evaluate the antioxidant and antihypertensive capacity. Lastly, the peptides present in the hydrolysates were identified using UHPLC-MS/MS.

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P54. A RAPID ELECTROKINETIC CHROMATOGRAPHY METHOD FOR THE CHIRAL SEPARATION OF 5-HYDROXYTRYPTOPHAN AND ITS APPLICATION TO THE ANALYSIS OF DIETARY SUPPLEMENTS

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Keywords: *electrokinetic chromatograhy, 5-hydroxytryptophan, Griffonia simplicifolia*, *dietary supplement*

5-Hydroxytryptophan (5-HTP) is a metabolite involved in the metabolic and neurological balance of the organism. It is produced by the hydroxylation of tryptophan (Trp) by two different enzymes: tryptophan hydroxylase 2 in the central nervous system and tryptophan hydroxylase 1 in peripheral tissues. Trp is an amino acid obtained only through dietary consumption which participates in neurological processes, stress, microbiota dysregulation, and depression. On the other hand, 5-HTP decarboxylation is necessary for the production of serotonin, an essential neurotransmitter that modulates vital biological processes such as sleep, circadian rhythm, appetite, mood, and learning. Alterations in 5-HTP physiological concentrations could be derived from diverse metabolic factors. One of them is Trp deficit due to low dietary intake, gut dysbiosis or alterations in tryptophan hydroxylases activity or production. As a consequence, 5-HTP and serotonin levels are related to pathologies such as anxiety, sleep disorders, depression or obesity [1,2]. Dietary 5-HTP supplementation based on *Griffonia simplicifolia* seeds extracts is commercially available and a popular treatment that improves positive emotion recognition [3]. Keeping in mind the chiral nature of 5-HTP, the quality control of 5-HTP-based dietary supplements is essential not only because the L-form of 5-HTP exhibits the desired biological activity but also because legal regulations do not allow the presence of the D enantiomer in these products.

In this context, this work was focused on the development of a rapid Electrokinetic Chromatography methodology enabling the chiral analysis of 5-HTP in dietary supplements. After evaluating the discrimination power of different cyclodextrins and the effect of the separation voltage and the temperature, a high enantiomeric separation (Rs 9.1) was obtained within 12 min using sulfated-γ-CD as chiral selector. To reduce the analysis time, a strategy based on the combination of a shorter capillary and a short-end injection was employed. Under these conditions, the 5-HTP enantiomers were separated in less than 5 min with a resolution of 4.7. Subsequently, the analytical characteristics of the developed methodology were evaluated and it was successfully applied to the quality control of a *Griffonia simplicifolia* dietary supplement rich in 5-HTP.

Acknowledgements

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P55. ENANTIOSELECTIVE DETERMINATION OF TRYPTOPHAN-RELATED METABOLITES IN URINE SAMPLES BY ELECTROKINETIC CHROMATOGRAPHY

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Keywords: *electrokinetic chromatography, tryptophan, kynurenine, 5-hydroxytryptophan, urine*

Tryptophan (Trp) is an essential chiral amino acid obtained exclusively through dietary intake that plays a critical role in physiological and neurological processes. It serves as a metabolic precursor for biologically significant compounds, including kynurenine (Kyn), 5-hydroxytryptophan (5-HTP), and serotonin; each of which fulfills distinct yet interconnected functions. Kyn pathway, responsible for the degradation of the majority of dietary Trp, is closely associated with immune response regulation, inflammation control, and neuroprotection.[1] Alternatively, a smaller portion of Trp undergoes hydroxylation to form 5-HTP, which is then decarboxylated to produce serotonin, a neurotransmitter fundamental to cognitive function, emotional regulation, sleep, appetite, and memory.[2] All the mentioned Trp-related metabolites, except serotonin, are chiral molecules. Disruptions in any of these Trp pathways have been involved in pathological conditions, including mood disorders, neurodegenerative diseases, and immune dysfunctions; highlighting the need for a comprehensive understanding of Trp metabolism and its downstream products.[3]

Enantioselective separations are especially valuable in fields such as pharmaceutical and biomedical analysis, where precise Chiral recognition is essential because the enantiomers of a single chiral compound may interact differently with the molecular receptors resulting in diverse biological effects. Capillary electrophoresis (CE) stands out as a highly powerful and versatile separation technique, offering advantages that make it well-suited for the resolution of enantiomers. In Electrokinetic Chromatography (EKC), the direct incorporation of a broad spectrum of chiral selectors into the background electrolyte, provides unmatched flexibility in method design and optimization; which is crucial for achieving enantiomeric separations.

In this study, the simultaneous enantiomeric separation of different metabolites involved in the tryptophan signaling pathway (Trp, Kyn, 5-HTP) and serotonin was carried out by an innovative approach based on EKC. Under the most appropriate experimental conditions, in terms of type and concentration of chiral selector, temperature, and voltage; the baseline resolution of all the target compounds was achieved in less than 20 min. Once the analytical characteristics of the developed methodology were evaluated, it was applied to the determination of the Trp-related metabolites in human urine samples. Before their analysis, urine samples were submitted to protein precipitation and solid-phase extraction for clean-up and preconcentration. The developed analytical strategy enabled the physiological detection of L-Trp and L-Kyn in the urine of a healthy volunteer.

Acknowledgements

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P56. COMBINING ELECTROCHEMICAL AND PROTEOMIC GROUNDBREAKING STRATEGIES FOR DETERMINING AND CHARACTERIZING CIRCULATING NUCLEOSOMES IN COLORECTAL CANCER SCENARIOS

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Keywords: nucleosomes, electroanalytical immunotechnologies, plasma, colorectal cancer, mass spectrometry, proteomics.

During cell death processes like apoptosis, nucleosomes — structures consisting of DNA, histones, and other associated proteins — are released into the bloodstream [1]. In this regard, detecting and analyzing circulating nucleosomes, their epigenetic modifications, and associated proteins offer a promising strategy for identifying non-invasive cancer biomarkers, improving early detection, prognosis, and disease monitoring, ultimately contributing to more personalized and effective cancer treatment [2].

In this context, a multilevel strategy has been developed for the first time for the determination of circulating nucleosomes in plasma using an anti-H3.1 histone variant, by coupling advanced proteomic analysis with a novel electrochemical bioplatform based on a sandwich immunoassay that uses magnetic beads as microsupports and amperometric detection on screen-printed carbon electrodes (SPCEs). The proposed electrochemical system enabled the detection and quantification of circulating nucleosomes. Its integration with mass spectrometry-based proteomics was used to confirm nucleosomes isolation and identify associated proteins with potential as colorectal cancer (CRC) biomarkers [3].

The immunotechnology showed an effective discrimination of the metastatic potential of CRC cells and was suitable for the quantification of H3.1 nucleosome expression levels in plasma samples from healthy individuals and patients with advanced CRC with outstanding analytical characteristics (LOD and RSD values of 7.82 ng mL^{-1} and 2 %, respectively) in a rapid, simple, sensitive, minimally invasive way and requiring minimal sample preparation. Furthermore, the analysis of isolated plasma nucleosomes by state-of-the art proteomics proved their specific isolation, with clear differences in their circulating levels between healthy individuals and advanced CRC patients. Additionally, the proteomics analysis allowed the identification of ten extracellular proteins, four of them described in databases as prognostic factors in other cancers different from CRC.

Importantly, the two technologies used in this multi-level approach feedback on each other, offering a multitude of future possibilities such as the exploration of other types of patient cohorts and the performance of longitudinal studies. Furthermore, the versatility of electroanalytical technologies for both the determination of proteins and their post-translational modifications, as well as epigenetic markers make them particularly well suited to detect and determine the clinical potential of these identified nucleosomal markers, and to implement multiplexed and multiomic technologies for their simultaneous determination in liquid biopsy samples, thus improving efficiency and accelerating the accurate and early diagnosis, prognosis, and therapeutic monitoring of complex and heterogeneous diseases such as cancer and even contributing to the design of personalized treatments.

Acknowledgements
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P57. SUSTAINABLE ALL-IN-ONE SILK FIBROIN-BASED IMMUNOPLATFORM FOR TIM-1 DETERMINATION IN ONCOLOGICAL SCENARIOS

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Keywords: *Silk fibroin, sustainability, Prussian Blue, electrochemical immunosensor, TIM-1, colorectal cancer.*

In the pursuit of novel, sustainable materials for biosensing applications, silk fibroin (SF)—a bioprotein extracted from *Bombyx mori* cocoons—has emerged as a tunable, ecofriendly and highly promising candidate [1]. While its application in enzymatic colorimetric sensing systems has been demonstrated, its potential in electrochemical biosensing remains largely underexplored [2].

In this work, we present the development of an SF-based immunoplatform for the electrochemical detection of T-cell immunoglobulin and mucin domain 1 (TIM-1), a clinically relevant biomarker implicated in cancer angiogenesis. The sensor architecture is based on the immobilization of capture antibodies (cAbs) within an SF matrix, taking advantage of its processability, biocompatibility, and capacity for structural tuning.

Detection was performed in a sandwich-type immunoassay format, employing a biotinylated detection antibody and streptavidin-conjugated horseradish peroxidase (HRP) for target recognition and signal generation. To enhance the analytical signal, Prussian Blue (PB), acting as an efficient redox mediator due to its excellent electrochemical reversibility, low overpotential for H_2O_2 reduction, and inherent biocompatibility, was strategically electrodeposited onto the disposable transducer surface. These features make PB an ideal amplifier for enzymatic signal transduction, particularly in systems utilizing HRP as label [3]. This novel integrated platform demonstrated sensitive and selective determination of TIM-1 standards and was successfully applied to the analysis of human plasma samples, enabling the stratification of patients with premalignant and early-stage colorectal cancer.

One of the key advantages of the proposed platform is its ability to maintain its functionality at ambient conditions for 20 days, eliminating the need for cold chain storage and significantly reducing associated costs. This is a particularly attractive feature in this type of biotool for ease of transport and use in resource-limited environments. In addition, the intrinsic tunability of SF - such as adjustable transparency and mechanical flexibility - opens the door to the development of fully customizable and robust biosensing platforms [4].

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P58. CUTTING-EDGE BIOSENSING PLATFORMS FOR THE EARLY DIAGNOSIS OF AUTISM SPECTRUM DISORDER

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Keywords: *mechanically interlocked derivatives of carbon nanotubes functionalized with alkene (MINTs-Al), biosensor, autism spectrum disorder (ASD), biomarkers*

In this work we present the design and development of cutting-edge methodologies to solve a significant current problem by multidisciplinary research, particularly focused on detecting new emerging biomarkers associated with autism spectrum disorder (ASD). The methodologies will be based on electrochemical biosensors, prepared using new mechanically interlocked derivatives of carbon nanotubes functionalized with an alkene (MINTs-Al) and Covalent Organic Frameworks (COFs). During the last years, the number of ASD individuals has dramatically increased and affects around 168 million people around the world, being 1 in 100 children. Effective early diagnosis methods are lacking, and therapeutic interventions are most effective if started promptly.T herefore, having methods that easily, and rapidly detect multiple emerging biomarkers associated with ASD will represent an advance in terms of diagnosing, stratify patients into subgroups, predict therapeutic response and design the most precise and effective treatments. Traditional methods for detecting biomarkers have limitations, since they require long analysis times, are expensive, hard to automate and require qualified personnel. This project proposes a competitive alternative that meet current demands (fast response, simple, low cost,miniaturize) and are industrially scalable. To do so, new hybrid materials designed to have specific properties, derived from COFs and new nanomaterials such as, will be combined with the most sensitive sensor technologies. It is worth tonote that the detection systems developed are so versatile, they can be applicable for the detection of other biomarkers associated with other disorders/ diseases by selecting suitable recognition reagents.

P59. ELECTROANALYTICAL STRATEGY TARGETING NON-CANONICAL G-QUADRUPLEX DNA STRUCTURES TO ADVANCE CANCER DIAGNOSIS AND THERAPY

V. Ruiz-Valdepeñas Montiel[a], A. Cabrero-Martín[a], R.M. Torrente-Rodríguez[a], E. Povedano[a], J. Velázquez-Gutiérrez[b], M. Garranzo-Asensio[b], J.M. Pingarrón[a], R. Barderas[b,c], S. Campuzano[a,c]

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Keywords: *electrochemical biotechnology, G-quadruplex, non-canonical structure, cancer.*

In the relentless pursuit of novel biomarkers for accurate cancer management, non-canonical (nc) DNA structures have emerged as compelling molecular signatures and promising therapeutic targets. While the classical double helix remains foundational to our understanding of DNA, nearly half of the human genome consists of repetitive elements, approximately 13% of which can adopt up to 15 distinct nc conformations that deviate from the classical model. These highly dynamic nc structures are involved in essential cellular processes such as DNA replication, transcription, translation, and genome stability, and have been linked to various diseases, particularly cancer.[1,2]

Among them, G-quadruplex (G4) motifs stand out due to their ubiquity across all domains of life and their multifaceted roles in transcriptomic regulation, microRNA biogenesis, mutational and epigenetic patterns, as well as their strategic localization in key oncogenic promoters and angiogenesis-related markers, which are associated with various cancer types. In fact, G4 deregulation has been documented in cultured cells, isolated chromosomes, tumor tissues, and even serum samples from colorectal cancer patients, underscoring their potential as biomarkers for accurate cancer diagnosis. Furthermore, their tunable stability and regulatory capacity position them as true molecular switches and highly attractive targets for oncologic therapy. [3-5]

In light of this background and the current lack of electrochemical strategies for G4 quantification, our research has focused on developing the first electroanalytical biotool for the rapid, sensitive, and cost-effective detection of these structures. In this communication, we will present the key principles of this innovative technology, which demonstrates excellent sensitivity for G4 motifs quantification both in synthetic sequences at nanomolar levels and in just 50 ng of genomic DNA extracted from cultured cancer cells. The platform also exhibits high specificity and ability to discriminate between cell lines with distinct metastatic potential or gene-silencing profiles. Inspired by the simplicity and functionality of glucometers, this technology addresses the limitations of existing G4 quantification methods by offering a portable, accessible, and scalable solution for point-of-care analysis, positioning itself as a transformative tool for truly democratized and precise oncology.

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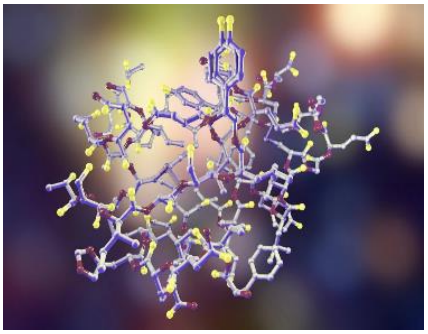

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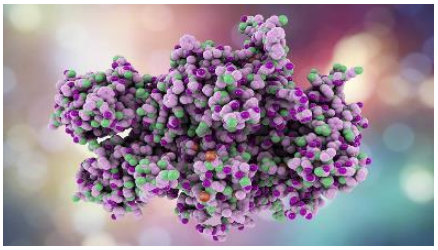

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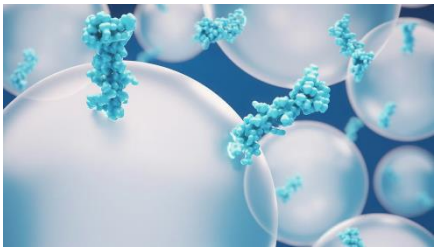

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BIOLAN Microbiosensores es una empresa biotecnológica que desarrolla, fabrica y comercializa biosensores enzimáticos, sensores potenciométricos y test inmunocromatográficos para la detección de parámetros relacionados con la calidad y seguridad alimentaria, así como con la salud. La estrategia de la compañía siempre ha sido la de **desarrollar tecnología propia**, basada en su plataforma tecnológica patentada de biosensores electroquímicos basados en enzimas, combinando conocimientos técnicos en electrónica, electroquímica y biotecnología.

BIOLAN en los últimos años ha estado inmersa en el proyecto de transformación digital, que ha dado como resultado una gama de productos digitalizados y dotados de conectividad, que permite la captura de los datos procedentes de los análisis llevados a cabo por los dispositivos y su posterior uso. Esta gama de productos está permitiendo avanzar a la compañía hacia una nueva etapa en la que el dato será el centro de su actividad, entendiendo que la digitalización de sus productos y sus propios procesos constituyen una oportunidad para la mejora de su competitividad.

Actualmente los principales segmentos del mercado alimentario al que se dirigen los productos de BIOLAN son : pesquero (BIOFISH HIS/YAKE), de los crustáceos (BIOFISH SUL), lácteos (BIOMILK LAC) , vitivinícola (BIOWINE) y Cárnico BIOMEAT (nitrito) .En 2024 lanza al mercado un producto para medir SAL(sodio) en cualquiera de los sectores antes mencionados . En cada uno de ellos se miden distintos parámetros: histamina, yake, sulfito, lactosa, ácido glucónico, ácido málico, azucares finales, nitrito , sodio etc.

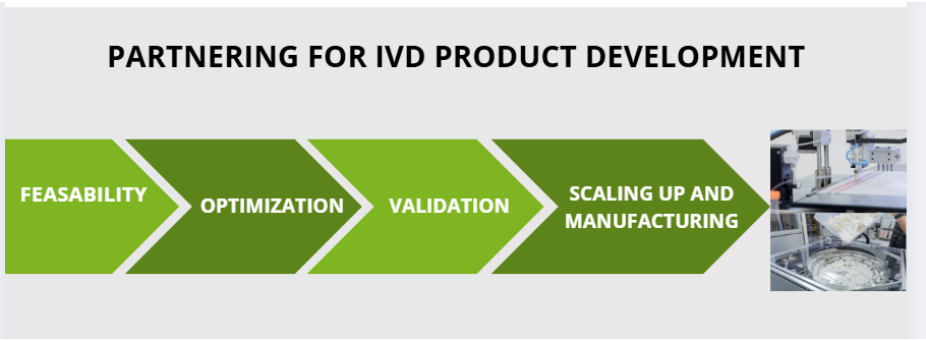
Pero además BIOLAN ha desarrollado productos dirigidos a la salud, como son: OSAXYL, dispositivo PoC para el diagnóstico de la hipolactasia (intolerancia a la lactosa), el test de antígeno para la detección de la infección por SARS-COV-2 y el test serológico para la identificación de la inmunidad ante el virus.

Además, en los últimos años, con el objetivo de dar respuesta a nuevas necesidades identificadas en el sector Salud, se ha lanzado una nueva Línea de Negocio basada en el desarrollo de sensores de presión para su aplicación diversos campos como el diagnóstico, la terapia y la rehabilitación**.**

Para poder abordar todos los retos que nos marcamos, contamos con un equipo científico-técnico que posee una dilatada experiencia en el desarrollo, el escalado y la producción de biosensores electroquímicos basados en electrodos serigrafiados, sensores de presión e inmunoensayos de Flujo Lateral con aplicación en el sector Alimentario o el sector Salud.

Por otro lado cabe destacar que contamos con las certificaciones ISO 9001: 2015 y desde 2023 la certificación ISO 14001 para la gestión ambiental , además de la ISO13485 y la licencia previa de funcionamiento otorgada por la AEMPS para fabricar y comercializar Productos Sanitarios.

En BIOLAN, además de desarrollar y producir productos propios, ofrecemos servicios de desarrollo personalizado a empresas, universidades y centros de investigación para llevar a cabo pruebas de concepto, escalados de prototipos, fabricaciones a pequeña y grande escala e incluso, certificado de producto sanitario.

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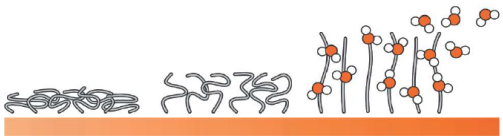

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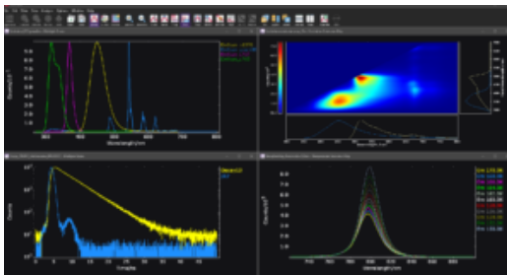
Raman ana Raman Confocal Spectroscopy

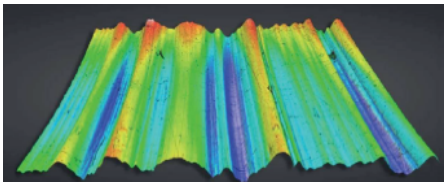
High end Spectroscopy Detectors

Advanced Spectroscopy Systems

Spectrometers: FTIR, Fluorescence, UV-VIS



SURFACE ANALYSIS



Non-contact 3D Optical profilers

Contact Angle Tensiometers

Potentiostats, Galvanostats


i+Med
COOPERATIVE
OF SCIENTISTS


BIOLAN
accurate · easy · smart


LASING


Scharlab
THE LAB SOURCING GROUP


Agilent
Trusted Answers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