Grupo de Ciencia y Tecnologías (Bio)Analíticas

Real Sociedad Española de Química

II Reunión Científica del Grupo Especializado en Ciencia y Tecnologías (Bio)Analíticas

Zaragoza, 27-28 de junio de 2023

Libro de Resúmenes

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II Reunión Científica del Grupo Especializado de Ciencia y Tecnologías (Bio)Analíticas

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

Samuel Bernardo Bermejo, María Castro Puyana y María Luisa Marina Alegre (Editores)

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Bienvenida

Estimados socios:

Celebramos la II Reunión Científica del Grupo Especializado de Ciencia y Tecnologías (Bio)Analíticas (GCTbA) de la RSEQ que da continuidad a la I Reunión celebrada hace un año en Granada.

Desde el Comité Organizador deseamos daros nuestra más cordial bienvenida a esta Reunión, así como agradeceros de nuevo todo vuestro apoyo y participación en las actividades del GCTbA. Este apoyo nos permitió crear este nuevo grupo cuyo objetivo ha sido y continúa siendo sumarnos a los esfuerzos de los químicos analíticos para potenciar la visibilidad y reconocimiento de la Química Analítica a nivel nacional e internacional tanto en los campos científico como social. Además, nos ha permitido continuar organizando actividades dirigidas a la consecución de nuestros objetivos, así como ampliar el número de socios del grupo que en la actualidad alcanza la cifra de 237.

Una vez más vuestra participación en esta II Reunión Científica ha superado las expectativas y se ha traducido en la posibilidad de organizar un Programa Científico intenso y de gran interés que incluye 2 conferencias plenarias, 28 presentaciones orales y 46 comunicaciones en formato poster. Siguiendo con nuestra política de apoyo a nuestros jóvenes investigadores, en esta Reunión todos los jóvenes doctorandos que han solicitado una presentación oral podrán realizarla (13 de las presentaciones orales) y todos ellos, hayan solicitado presentación oral o poster, podrán optar a los distintos premios que se concederán a las mejores comunicaciones presentadas en cualquiera de dichos formatos en el marco de nuestra Reunión. Para ello, los Jurados designados al efecto desarrollarán su labor durante nuestra Reunión con el fin de hacer posible otorgar los premios a las comunicaciones más destacadas. Como novedad con respecto a la I Reunión Científica, y atendiendo a vuestros comentarios en dicha Reunión, este año el Programa Científico incluye una mesa redonda dedicada a la Información Químico-Analítica: Calidad e Impacto Científico y Social en la que todos los asistentes podrán aportar sus opiniones y comentarios durante el debate que tendrá lugar sobre un tema candente que nos afecta a todos. Para dar cabida a este Programa Científico la duración de esta II Reunión Científica se ha ampliado con respecto a la del año pasado suponiendo ello un aliciente para continuar con nuestra tarea de seguir reforzando y ampliando nuestras actividades y esfuerzos para conseguir los objetivos marcados.

Es un hecho que las actividades de transferencia constituyen una prioridad para los químicos analíticos y que forma parte de nuestra vocación por dar soluciones a diferentes problemas de interés científico y social en los distintos ámbitos: salud, alimentos, medioambiente, sostenibilidad, etc. En esta actividad juega un papel muy relevante el sector productivo como se pone de manifiesto en la participación de distintas empresas que patrocinan esta II Reunión Científica: Perkin Elmer, Gold Standard Diagnostics Madrid, Lasing,

Metrohm Hispania y Microx Technologies. Además, contamos con el patrocinio del Journal of Analytical Atomic Spectrometry, Microchimica Acta y Analytical & Bioanalytical Chemistry. A todos ellos nuestro agradecimiento por su inestimable contribución a esta Reunión Científica. Gracias también al Ayuntamiento de Zaragoza, a la Universidad de Zaragoza, y a la Universidad de Alcalá y su Fundación General por contribuir apoyando la organización de nuestra Reunión Científica.

En el contexto de esta II Reunión Científica celebraremos también la II Asamblea de Socios del GCTbA y se hará entrega de los Premios concedidos a los ganadores de la Segunda Edición de los Premios GCTbA a la mejor Tesis Doctoral y a la Transferencia de Tecnología. Nuestra más cordial enhorabuena a los galardonados.

Todos habéis seguido los distintos homenajes dedicados a la memoria de los compañeros que nos han dejado recientemente y que han tenido lugar en el contexto de distintas reuniones científicas relacionadas con la Química Analítica que se han celebrado a lo largo del último año. Nuestro agradecimiento y reconocimiento seguirá presente en esta II Reunión Científica del GCTbA. Nuestro mejor homenaje es recoger su legado y continuar con el camino que ellos dejaron trazado.

Os deseamos una muy fructífera y agradable estancia en Zaragoza y que esta II Reunión Científica del GCTbA contribuya a generar nuevas oportunidades de colaboración y de avance para nuestra disciplina.

María Luisa Marina

Presidenta GCTbA

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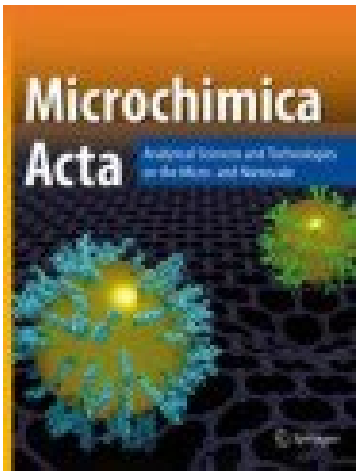

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MARTES 27 DE JUNIO DE 2023

8:30 h	Acreditación, entrega de documentación y colocación de pósters
8:45	**INAUGURACIÓN DE LA II REUNIÓN DEL GCTbA**
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9:45	***CONFERENCIAS ORALES: SESIÓN 1*** ***Moderadores: S. Campuzano, J.M. Costa***
9:45	**O1 Supramolecular solvents: making comprehensive sample treatment in multiclass analytical methods** *N. Caballero-Casero, S. Rubio*
10:05	**O2 Molecularly Imprinted Polymers as versatile Materials for analytical applications** *J. González-Rodríguez*
10:25	**O3 G-quadruplex-based DNAzyme for sensitive biomarker detection with the naked eye** *B. Martín, L.A. Tortajada-Genaro, A. Maquieira*
10:45	**O4 Tetrahedral DNA nanostructures combined with few-layer bismuthene for rapid and sensitive virus detection** *E. Lorenzo, L. Gutiérrez-Gálvez, D. García-Fernández, M. del Barrio, M. Luna, I. Torres, F. Zamora, M. Castellanos, A. Somoza, F. Pariente, T. García-Mendiola*
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12:00	***CONFERENCIAS ORALES: SESIÓN 2*** ***Moderadores: A. Ríos, M. Castro-Puyana***
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12:15	**O6 Matrix metalloproteinases and tissue inhibitors of metalloproteinases influence in breast cancer** *S. Escudero-Cernuda, R. González de Vega, M. Fraile, N. Eiró, F. Vizoso, M.L. Fernández Sánchez*
12:30	**O7 Tetrazole hybrids targeting beta-amyloid and metal ions in Alzheimer's disease** *D. Vicente-Zurdo, M. Cubo-Pareja, S. Rodríguez-Blázquez, N. Rosales-Conrado, M.E. de León González, J. Domingo Sánchez, J.C. Menéndez, J.F. González, Y. Madrid*
12:45	**O8 Salivary estradiol as biomarker for the evaluation of the menstrual cycle: fact or MYTH?** *D. Fabregat-Safont, J. Fabregat-Nabás, A. Gomez-Gomez, N. Haro, E.R. Velasco, R. Andero, O.J. Pozo*
13:00	***CONFERENCIAS ORALES-JÓVENES*** ***Moderadores: A. Escarpa, M.T. Fernández-Abedul***
13:00	**OJ1 Tracking lesser-known allergenic proteins and carbohydrates with electrochemical bioplatforms** *M. Blázquez-García, V. Serafín, V. Ruiz-Valdepeñas Montiel, S. Benedé, E. Molina, M. Gamella, B. Arévalo, L. Mata, P. Galán-Malo, I. Segura-Gil, J.M. Pingarrón, S. Campuzano*
13:10	**OJ2 Nanoparticle-based enhacement in nanochannel sensors for monitoring enzymatic cleavage: application to MMP-9 detection** *D. Valero-Calvo, C. Toyos-Rodríguez, F.J. García-Alonso, A. de la Escosura-Muñiz*

13:20	**OJ3 Rapid screening of novel antimicrobial compounds with a nanoporous-based electrochemical platform** *C. Toyos-Rodríguez, D. Valero-Calvo, K. Ivanova, T. Tzanov, L. Vilaplana, M. Pilar-Marco, A. de la Escosura-Muñiz*
13:30	**OJ4 Use of gold nanoparticles to develop a smartphone-based point-of-care biosensor for prostate specific antigen ultrasensitive quantification** *G. Redondo-Fernández, L. Cid-Barrio, M.T. Fernández-Argüelles, A. de la Escosura-Muñiz, A. Soldado, J.M. Costa-Fernández*
13:40	**OJ5 Using complementary analytical techniques for the study of protein corona formation onto PtNPs in different biological media** *A. López Gutiérrez, N. Rodríguez-Fariñas, R.C. Rodríguez Martín-Doimeadios*
13:50	**OJ6 Enzymatic synthesis of nanomaterials as indicators in optical biosensors** *J. Camacho-Aguayo, S. de Marcos, J. Galbán*
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17:10	**O12 Neuroprotective potential of bioactive compounds from avocado residues** *E. Gómez-Mejía, D. Vicente-Zurdo, N. Rosales-Conrado, M.E. León-González, Y. Madrid*
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10:05	**O14 Authenticating bee pollen origin by using different analytical techniques** *A.M. Ares, B. Martín-Gómez, J.A. Tapia, L. Toribio, M.T. Martin, J. Bernal*
10:25	**O15 Fluorescein-gold nanoparticles fret-based detection of miRNA using nucleic acid enzymes as amplification tool** *M.T. Fernández-Argüelles, A. Sánchez-Visedo, A. Soldado, L. Royo, F.J. Ferrero, J.M. Costa-Fernández*
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Premios

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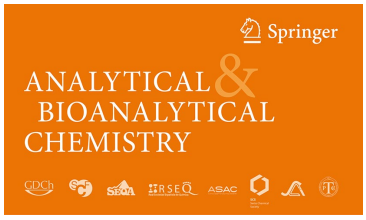

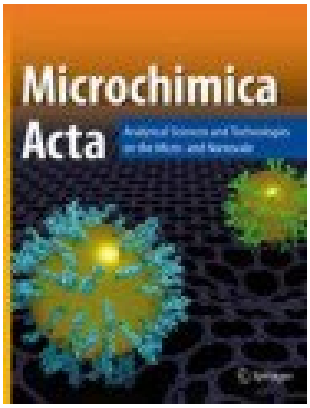

Conferencias plenarias

PL1. ELECTROCATALYTIC PALLADIUM NANOCLUSTERS FOR SENSITIVE BIOASSAY DETECTION: IMMUNOASSAY OF A STROKE BIOMARKER AND DETECTION OF ISOTHERMAL AMPLIFICATION OF SARS-COV-2 GENETIC MATERIAL

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Keywords: *palladium nanoclusters (PdNCs), electrocatalysis, loop-mediated isothermal nucleic acid amplification (LAMP), competitive immunoassay, Glial Fibrillary Acidic Protein, SARS-CoV-2.*

There has been a relevant breakthrough in bioanalytical chemistry due to the implementation of nanotechnology in traditional analytical techniques, such as enzyme-based immunoassays with colorimetric detection or gene amplification by polymerase chain reaction (PCR). In this context, metal nanoclusters (MNCs) seem to be a very interesting alternative to nanoparticles (NPs) due to their smaller size, which allows labelling antibodies with little loss of activity[1].

PdNCs have been synthesized using two different synthesis methodologies obtaining different sizes. The catalytic activity of the smaller on the oxygen reduction reaction has been used to carry out the determination of SARS-CoV-2 by means of isothermal genetic amplification and the change in pH that occurs[2]. On the other hand, the larger PdNCs showed catalytic activity on the hydrogen evolution reaction, that were employed for the bimodal determination of GFAP, a biomarker that allows differentiating between ischemic and hemorrhagic strokes. This was carried out by means of a competitive immunoassay that used specific antibodies labelled with PdNCs, whose catalytic effect was monitored voltammetrically and using elemental mass spectrometry.

In summary, PdNCs have been shown to be electrocatalytic markers that can be used to develop highly sensitive bioanalytical methodologies combined with e.g., immunoassays or isothermal amplification reactions.

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PL2. SCREENING OF EMERGING CONTAMINANTS IN WATERS BY COMBINED USE OF GAS AND LIQUID CHROMATOGRAPHY HYPHENATED TO HRMS

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Keywords: *wide-scope screening, pesticides, pharmaceuticals, aquatic environment, detection, identification, chromatography-high resolution mass spectrometry.*

The complexity of the aquatic environment, including urban wastewater, together with the huge number of organic micropollutants (OMPs) that may be present in the samples, makes the comprehensive investigation of these compounds an analytical challenge. Pesticide residues are usually present in many water samples around the world, particularly in agricultural areas. In the last years, contaminants of emerging concern, mainly pharmaceuticals, are also frequently investigated in the aquatic environment due to their ubiquitous presence associated to their widespread consumption and incomplete removal in conventional wastewater treatment plants. Currently, one of the most powerful analytical techniques for wide-scope screening of water samples is High Resolution Mass Spectrometry (HRMS), making use of different approaches (e.g. target, suspect and non-target analysis). Considering the different physico-chemical characteristics of OMPs, the coupling to both liquid (LC) and gas chromatography (GC) to HRMS is indispensable in order to widen the scope of the screening, from volatile, non-polar compounds, to semi/non-volatile, polar compounds[1].

In this work, the potential of LC and GC coupled to HRMS using Orbitrap and/or QTOF analyzers is discussed for screening of pharmaceuticals and pesticides in surface water and wastewater samples. Different strategies are presented in both LC and GC-HRMS based screening (e.g. use of MS^E in LC-HRMS; EI versus APCI sources in GC-HRMS, etc), and illustrative examples are given, including detection and identification of metabolites/transformation products. The searching was made by target (standards available) and suspect (without standards) approaches, using home-made databases containing more than 1500 compounds (LC-QTOF MS)[2] and around 500 pesticides (GC-QTOF). The (tentative) identification was possible at different confidence levels, as a function of the standards availability and the accurate-mass information obtained for the molecular ion/protonated molecule (generally in the low energy function (LE)) as well as for the fragments (typically observed in high energy function (HE)), and after evaluation of whether the potential fragments are consistent with the chemical structure of the compound[3]. Ion mobility adds an extra dimension separation, improving the quality of mass spectra, especially in HE. In addition, the use of CCS values (both experimental and predicted) increases the reliability of the identification process[4].

In summary, the power of chromatographic separations together with the excellent characteristics of HRMS (high resolution, full spectrum accurate-mass data) allows to investigate thousands of compounds in the samples, without the need of having all the reference standards available. In this way, data provided by HRMS screening can be used to focus subsequent analytical efforts on the most relevant compounds identified in the screening (e.g. monitoring campaigns for application of quantitative methods based on GC-MS/MS and/or LC-MS/MS).

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Comunicaciones invitadas-
Premios GCTbA

OI1. KNOWLEDGE TRANSFER WITHIN ATOMIC SPECTROSCOPY. IS IT POSSIBLE?

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Keywords: *inductively coupled plasma spectroscopy, universal analysis, interferences, sustainable analysis laboratory.*

Last year, a modification of the 2011 Spanish Law for Science, Technology, and Innovation was approved and published[1]. A key point considered by this law is the transfer of the knowledge generated from fundamental research to society. Obviously, basic research is required to find new solutions to emerging problems and improve the performance of existing technology. On this subject, Spain meets the highest standards in basic scientific research. Nevertheless, eventually, productive ecosystems will lack access to the knowledge that research laboratories produce.

It is recognized that, according to the global indicators of technology transfer (e.g., patents, spin-off generation, collaborations with private companies, etc.), Spain must improve the level of cooperation between private institutions and public research organizations. Efforts have been made in order to promote technology transfer[2], but much more needs to be done on this subject.

Within this context, researchers must decide whether to remain in their laboratories doing good science or to go further and try to implement their findings in a real-world context. However, some research topics are apparently too far from reality, or they seem to be exclusively related to pure knowledge without a clear application to the real world.

Possible subjects exemplifying the above-mentioned situation could be: studying interferences in inductively coupled plasma techniques; methods for overcoming these unwanted phenomena; evaluating the plasma compatibility with organic chemicals; lowering the sample amount required to perform the analysis; or improving analytical figures of merit. What is the link between the results obtained in these areas and companies? How can we export these achievements to private institutions? What are the real benefits of this research in terms of value generation? Which instruments are available to carry out this transfer effectively? How can the results be exported to the industry? These are questions that must be answered in order to take full advantage of our research and, besides, to contribute to the fulfillment of the third mission of our university: knowledge transfer to society.

All these points will be discussed in the present talk, which will combine scientific discussions as well as comments aimed at helping young researchers catalyze knowledge transfer. In the first case, an overview of how the different findings led us to design solutions that finally provided a compact solution will be given. Possible strategies to finally implement the knowledge acquired within the frame of the studies will be discussed, including the tools that are available in our universities at the regional and national levels.

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OI2. MAD-SAN ROUND TRIP: 9393 KM POWERED BY STATE-OF-THE-ART ELECTROCHEMICAL BIOSENSORS

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Keywords: *food safety, personalized nutrition, foodomics, electrochemical biosensor.*

Almost 9 years ago, my first contact with electrochemical biosensors pushed me to do my PhD Thesis, the best personal and professional journey of my life. A round trip from the Electroanalysis and Electrochemical (Bio)sensors Group at the Universidad Complutense de Madrid, my *"alma mater"*, to the Nanobioelectronics Laboratory at the University of California San Diego, led by Prof. J. Wang, including stops at the ProteoFUN, Plant Biotechnology, Protein Structure-Function Groups, and ZEULAB S.L. and DSM Nutrition companies. Although the original roadmap only covered food safety, the use of state-of-the-art electrochemical bioplatforms and their application to the agri-food and clinical areas soon launched me into the set-up of bioelectronic devices for personalized nutrition, and I am currently continuing to advance one of the most exciting approaches, foodomics.

Although the key role of nutrition in health and well-being is now fully accepted, further advances in agri-food tech and dietary and nutritional surveillance are still required to prevent and treat diseases such as diabetes, allergies, and chronic nutritional disorders, as well as to respond to new challenges, such as climate change, access to sustainable land and water, rising inflation, and war-related food shortages. Therefore, the main objective of my PhD Thesis was the development of electrochemical bioplatforms for the reliable biosensing of food and clinically relevant analytes in poorly treated highly complex samples. And we achieve this by rationally exploiting the use of disposable electrochemical platforms with: (i) magnetic microcarriers as solid supports for the development of immune- or nucleic acid- bioassays for the detection with the required sensitivity and selectivity of the allergens that affect children the most or cause the most severe reactions, as well as some of the most significant adulterations, such as the detection of animal species different from those declared in milk and meats, and ii) transient polymeric coatings as electrode modifiers, for the protection of (bio)sensing surfaces during prolonged incubation fouling biological medium and/or denaturing pH values[1].

Apart from food interrogation, the assessment of its interplay and impact on human organism is crucial to guide individualized nutrition. In this regard, although several digital and physical wearable sensor approaches for nutrition monitoring have been reported, real-time quantification of nutrition-related biochemical markers using hand-held devices has hardly been implemented. Consequently, one of the topics of my postdoctoral period focused on the design of bioelectronic or 3D printing-based devices for diabetes and personalized nutrition surveillance using electrochemical chips for on-the-spot self-testing of insulin or vitamins to strengthen the immune system and intestinal microbiota[2].

My ongoing research is aimed both at the development of state-of-the-art electroanalytical devices for the n-plex detection of multiomics (bio)markers (proteins, nucleic acids, oligosaccharides) associated with allergenic processes or nutritional disorders directly in real animals or incurred samples or in organelles isolated from them and little explored with this type of biodevices, as well as their implementation in all-in-one electrochemical reading tests for simple and fast sampling to advance in foodomics.

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OI3. TOWARD BIOSENSING SYSTEMS BASED ON FLUORESCENT PEROVSKITE NANOPARTICLES

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Keywords: *metal halide perovskite nanocrystals, fluorescent labels, biosensing, bioimaging.*

Metal halide perovskite (MHP) nanocrystals (NCs), with formula $APbX_3$ (A = Cs^+, $CH_3NH_3^+$; X = Cl^-, Br^-, I^-), have been spotlighted in the last decade due to several optical features, which converts this material into a highly cost-effective candidate for developing fluorescent labels in biosensing and bioimaging[1]. Besides their high photoluminescence quantum yields, narrow emission bands, broad absorption bands, multiphoton absorption cross-sections, and especially, an easily tunable emission spectrum, offering a wide colour gamut, some drawbacks like their fast degradation in polar media or the spontaneous coalescence of colloidal NCs still limit their use in biological applications[1].

In this communication, we present three strategies for obtaining water-stable fluorescent labels based on perovskite nanocrystals, and their utilization in biosensing approaches (**Figure 1**). In strategy A, the perovskite NCs were crystallized inside mesoporous silica (MS) particles, with a post-synthetic heat treatment which conferred water-stability to the nanoparticles[2]. In Strategy B, the NCs were generated by water-induced phase transformation from APb_2X_5 to $APbX_3$, obtaining core-shell particles in the presence of a silica source, with a further heat treatment[3]. Strategy C provided sealed polymeric nanocapsules containing colloidal NCs, formed through a solvent-antisolvent approach, according to previously reported methods[4]. Multicoloured nanoparticles were synthesized by varying the halide composition, preserving photoluminescence after encapsulation. The inorganic type A labels were successfully employed as contrast agents for staining mouse cells, and the results were comparable to commercial dyes. As a first approximation for detecting drug allergies, anti-immunoglobulins-E were conjugated to type B labels and later selectively recognized by a specific receptor immobilized on a polystyrene surface. Anti-lactate-dehydrogenase antibodies were labelled with type C nanoparticles and selectively recognized by LDH protein immobilized on a polystyrene plate. Type C labels performance for lateral-flow immunoassay is also being studied with an IgG-antiIgG model system. This line of research paves the way toward highly sensitive and multiplexed biosensing systems based on easily-obtained and water-stable fluorescent labels.

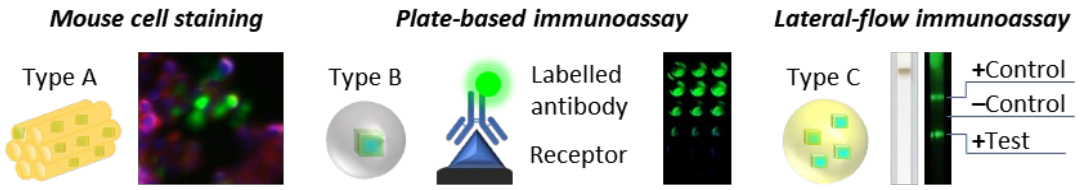

Figure 1. *Fluorescent labels based on stabilized perovskite nanoparticles for biosensing approaches.*

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FI4. ANALYTICAL NANOSCIENCE AND NANOTECHNOLOGY APPLIED TO THE FOOD INDUSTRY: DESINING NANOMICELLES TO INCREASE VITAMIN D3 BIOAVAILABILITY

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Keywords: *nanomicelle, vitamin D3, self-assembly, nanometrology, fluorescence, doped graphene quantum dots.*

Functional foods provide extra benefits to ensure and improve public health. They could be produced by fortification, applying bioactives like vitamins. However, due to their unstableness and limited water solublity, the bioavailability of bioactive ingredients can decrease during processing, storage or ingestion. Nowadays there is an emerging trend within the food industry based on the development of different nanoencapsulation systems with the aim to solve the above cited issues. Among different strategies, it should be highlighted the synthesis of nanomicelles (NMs) using self-assembly methods, mainly because of its easier implementation and required inexpensive equipment. Vitamin D3 is a fat-soluble bioactive, becoming the object of novel research lines due to its role displayed in mineral metabolism especially associated with calcium and phosphorus, diabetes prevention, hypertension and multiple sclerosis. Though, its sensitivity to light, heat and oxygen has limited its implementation as nutraceutical in foods applications[1].

In this study vitamin D3 loaded nanomicelles (VD3NMs) were developed for the first time and its physicochemical and release behavior were studied. After a detailed optimization procedure attending to the obtained encapsulation efficiency (EE), polydispersity index (PdI) and droplet size results, the suitable final composition for VD3NMs was: 0.5 mM surfactant mixture (0.45 mM of tween 60 and 0.05 mM of saponin quillaja) and 0.25 mM of cholecalciferol in MES 10 mM at pH 7.7. Physic-chemical and nanostructural characterization were carried out by DLS, ELS, UV-Vis and Raman spectroscopies and Scanning Electron Microscopy (SEM). By DLS, it was obtained a VD3NMs nanoparticle diameter of 49.63 ± 2.24 nm (n = 9). The UV-Vis spectra obtained for free VD3 shows the typical absorption bands at 211 and 263 nm. The absorption spectra of VD3NMs provides a single band at 253 nm, which allowed us to discriminate between free VD3 and encapsulated. EE was determined by UV-Vis spectroscopy being of 90 ± 1.5%. The Raman spectra shows a band at 1649 cm^{-1} when the bioactive is unencapsulated whereas when it is encapsulated a wider band appears. SEM reported a spherical shape morphology with a physical diameter of 35.7 ± 10.4 nm.

Moreover, a selective and sensitive sensing fluorescent nanoprobe based on quantum dots co-doped with S and N atoms was synthesized for both detection and quantification of free and VD3 nanomicelles based on their photoluminescent quenching at rising analyte concentrations[2]. Eventually, this research was implemented to perform analytical characterization of commercial nutraceutical supplements containing native encapsulated VD3 and non encapsulated VD3.

Acknowledgements
This work was supported by Spanish Ministry of Science and Innovation [grant number PID2019-104381GB-I00] and by JJCC Castilla-La Mancha [grant number JCCM SBPLY/21/180501/000188].

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Comunicaciones orales

O1. SUPRAMOLECULAR SOLVENTS: MAKING COMPREHENSIVE SAMPLE TREATMENT IN MULTICLASS ANALYTICAL METHODS

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Keywords: *supramolecular solvents, multiclass analytical methods.*

Multiclass analytical methods have become relevant for control laboratories that are routinely confronted with hundreds of substances for which maximum permitted levels have been set and decisions on positive and negative samples have to be taken quickly (e.g. agrifood or anti-doping labs). Multiclass analytical methods are also relevant for environmental monitoring, epidemiological studies, exposomics, metabolomics, etc. where detection of as many toxics as possible in a single analysis is highly valuable.

Both high and low resolution mass spectrometry, particularly combined with liquid chromatography are currently the techniques of choice for multiclass analytical methods. These methods should be comprehensive, and given the huge number of analyses usually required, they should allow high throughput sample processing and be cost-effective and green. In this respect, one of the greatest challenges of multiclass analytical methods is how to extract efficiently the targeted substances and how to reduce sample matrix effects while preventing the loss of chemicals with very different physicochemical properties.

Because of the distinctive features of supramolecular solvents (SUPRASs), their application in the development of multiclass analytical methods is deserving closer attention in the last few years[1]. Thus, the different polarity microenvironments present in the amphiphilic nanostructures of SUPRASs render them excellent candidates for the extraction of analytes in a wide polarity range. By their own nature, SUPRASs are non-selective extractants, as required in multiclass analytical methods, but they can be tailored to remove major matrix macrocomponents (e.g. proteins, humic acids, carbohydrates, etc.), which reduces interferences and variability among samples. On the other hand, the high number of binding sites derived from the large concentration of amphiphile in the SUPRAS, together with the mixed mechanisms offered for solute solubilisation, allow efficient extractions using low SUPRAS volume, which makes unnecessary sample extract evaporation.

This presentation deals with the main achievements obtained so far, as well as the challenges ahead, regarding the application of SUPRASs to the development of comprehensive sample treatments in multiclass analytical methods. Major achievements have been related to the progress made in the tailoring of SUPRASs, which has permitted to get solvents fit-for-purpose. To illustrate achievements, we will focus on two areas of analysis; environmental epidemiology[2] and anti-doping control[3]. In the first one, SUPRAS have been successfully used for determining the occurrence of a wide variety of multiclass emerging pollutants in both biological human samples (urine, serum, saliva, hair, nails, sweat) and human exposure sources (food, beverages, drinking water, dust). A valuable asset of SUPRASs in this area is their ability for developing matrix-independent methods since exposure evaluation always involves the analysis of very different types of samples. Concerning anti-doping control, SUPRASs have been successfully applied to the screening and confirmation of around a hundred of prohibited substances, categorized by the World Antidoping Agency in 10 structural groups, which covered a wide polarity range (log P -2.4 to 9.2). In this way, a single method can replace three different methods with a narrower analyte scope in sample treatment. The challenges to be confronted in the application of SUPRASs in multiclass analytical methods, which will require further developments, will be discussed.

Acknowledgements
Authors thank the funding to MCIN/ AEI /10.13039/501100011033. N. Caballero-Casero thanks for her postdoctoral grant (IJC2020-044941-I/AEI/10.13039/501100011033.

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O2. MOLECULARLY IMPRINTED POLYMERS AS VERSATILE MATERIALS FOR ANALYTICAL APPLICATIONS

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Keywords: *molecularly imprinted polymers, sensors, filters, smart-materials, high-selectivity.*

The first attempts to establish Molecularly Imprinted Polymers (MIPs) were developed in the first half of the twentieth century, with greater development in the decade of the 1980's and 1990's. Since then, there has been wider use of these in a range of applications across many fields, from engineering to biomedical applications. The concept has also been widely exploited in analytical chemistry, as the concept of materials highly selective for a range of analytes or a single molecule is quite attractive for a range of analytical applications. From chromatographic separations, as a pre-treatment technique to enhance selectivity, to the design of different types of sensors, where a bonus might be achieving a lower limit of detection, these materials offer great versality. Also, the simplicity of manufacturing together with the offer of a wide range of materials with different properties to build them and opportunities for functionalisation are positioning them in an advantageous position compared to other competitor materials. Its use as a smart material with stimulus-driven response abilities are also making them future-proof for some years to come. This talk will offer a quick overview of applications in different fields of analytical chemistry, especially in the areas of security and forensic science[1,2], industrial separation[3] and environmental control[4,5]. It will also indicate future trend of use for them.

Acknowledgements
University of Lincoln (UK) for academic and research support.

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O3. G-QUADRUPLEX-BASED DNAZYME FOR SENSITIVE BIOMARKER DETECTION WITH THE NAKED EYE

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Keywords: *optical transduction, aptamer, biosensor.*

Biosensors offer reliable detection of many biological and chemical substances with high selectivity and sensitivity, small size, and cost-effectiveness. Currently, the last step of many biosensing systems is signal amplification and the conventional approach uses enzymes, such as horseradish peroxidase or alkaline phosphatase. As an alternative, certain nucleic acids have attracted increasing interest because they also exhibit catalytic properties and show some advantages compared to traditional protein enzymes. These DNAzymes have a unique ability to organize into various non-canonical structures. A relevant example is a four-stranded DNA structure called the G-quadruplex, which has been used for diagnostic and therapeutic applications. In recent years, the hemin/G-quadruplex complex has been described in electrochemical, colorimetric, and chemiluminescence sensors for detecting targets such as metallic ions, organic molecules, and proteins[1,2].

This study presents the advances for determining biomarkers support on aptamers as biorecognition elements and G-quadruplex structures as the signal amplification system. Analytical performances were successfully compared to immunoassay staining and enzymatic amplification. As a proof-of-concept, the determination of serum biomarkers has been approached. DNA molecules were designed to integrate both target recognition and signal transduction. The tested substrates were ABTS and luminol for colorimetric and chemiluminescence transduction. The solution was a sensing system enabling fast and specific naked-eye detection. Thus, the approach can be helpful for accurate diagnostics and support effective clinical care.

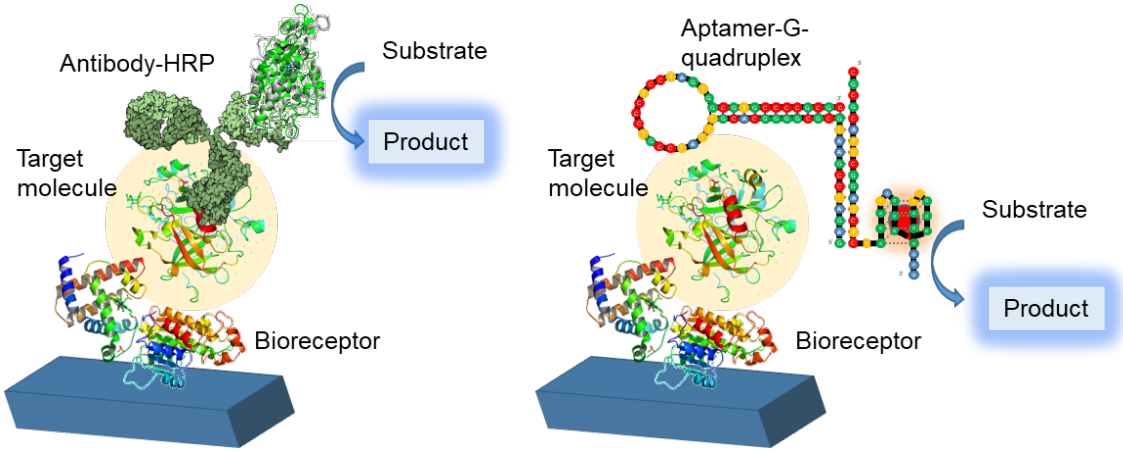

***Figure 1**. Scheme of biosensing systems based on: (left) immunoassay staining and enzymatic amplification, (right) aptamers with G-quadruplex structures.*

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O4. TETRAHEDRAL DNA NANOSTRUCTURES COMBINED WITH FEW-LAYER BISMUTHENE FOR RAPID AND SENSITIVE VIRUS DETECTION

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Keywords: *biocatalyzed ECL, virus, SARS-CoV-2 detection.*

A biocatalyzed electrochemiluminescence (ECL) biosensor based on few-layer bismuthene (FLB) and tetrahedral DNA nanostructures (TDNs) for virus detection is presented. FLB has been used as immobilization platform of the biorecognition agent (an oligonucleotide complementary to the analyte) included in one of the vertices of the TDNs. The other three vertices carry three thiol groups, that allow TDNs immobilization on the FLB surface.

Briefly, as it is depicted in the scheme of **Figure 1**, the biosensing platform consists of a carbon screen-printed electrode (CSPE) nanostructured with FLB (step 1) and modified with TDNs (step 2). As case of study, we have chosen as virus target the SARS-CoV-2. Therefore, TDNs contain, as capture probe, a specific SARS-CoV-2 gene region (ORF1ab) in one of its vertices (TDN-ORF). DNA target sensing takes place by the hybridization of the DNA analyte, a specific sequence of (ORF-C) with the capture probe (step 3). Detection of hybridization event and amplification of the biosensor response is carried out by a second hybridization step with a biotinylated reporter probe (ORF-2-Biotin) (step 4), which allow the further immobilization of an avidin-glucose oxidase conjugate (Av-GOx) (step 5). The addition of glucose generates H_2O_2, which in presence of luminol (step 6) gives the ECL response (step 7). To assess the applicability of the developed biosensing platform, it was successfully applied to the detection of SARS-CoV-2 in nasopharyngeal samples from infected patients without any amplification process. Thus, it can be a good alternative tool for COVID-19 diagnosis.

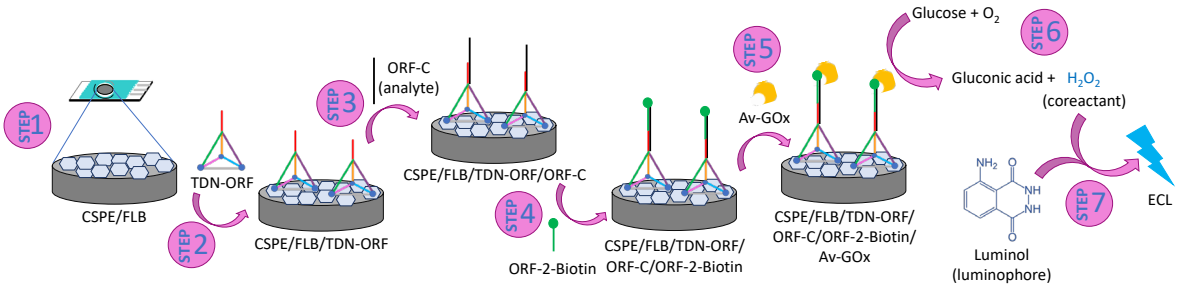

Figure 1. *Biocatalyzed ECL platform for SARS-CoV-2 detection.*

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O5. EARLY AND DIFFERENTIAL DIAGNOSIS OF CHRONIC DISEASES BY MULTIPLEXED ELECTROCHEMICAL INTERROGATION OF THE HUMORAL INMUNE RESPONSE

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Keywords: *autoantibody, autoimmunity, electrochemical immunosensor, multiplexed.*

Humankind's struggle to find the elusive cure for chronic diseases (CDs) among systemic lupus erythematosus (SLE), Sjögren's syndrome (SS), and Alzheimer's disease (AD) highlights the continuing need to develop new innovative diagnostic tools, making an ongoing therapeutic priority the identification of new molecular targets to fight against. The humoral immune response is now considered to have much to offer in the minimally invasive, early, and reliable diagnosis and in minimizing the terrible effects associated with these increasingly prevalent CDs through the identification and validation of molecular signatures of specific autoantibodies (Abs).

To this end, our most recent research has addressed the development of multiplexed electrochemical bioplatforms on disposable screen-printed electrodes to contribute to the early and reliable diagnosis of autoimmune and neurodegenerative diseases by identifying and demonstrating the diagnosis potential of quadruple Abs signatures comprising those produced against extractable nuclear antigens (ENAs): La/SSB-Abs, Ro/SSA-Abs, U1snRNP70-Abs, and smRNP-Abs[1] and the individual and total content of the three most common isotypes (IgGs, IgMs, and IgAs,) of dsDNA-Abs[2]. Both bioplatforms took advantage of the use of commercially available magnetic microcarriers that we modified with the specific nuclear antigen using His-tag, or EDC/NHS covalent chemistries, or with a biotinylated DNA prepared in the laboratory from a human plasmid exploiting the biotin-streptavidin interaction. Captured specific autoantibodies were enzymatically conjugated with HRP-labeled secondary antibodies and detected by amperometric transduction after trapping of the magnetic bioconjugates on the working surfaces of disposable quadruple platforms. The variation of the cathodic currents recorded (–0.20 V vs. Ag reference pseudoelectrode) in the presence of hydroquinone (HQ) and H_2O_2 was directly proportional to the concentration of each of the target Abs.

The developed methodologies exhibited high analytical performance in terms of sensitivity, selectivity, simplicity, and rapid response compatible with their clinical applicability for multiplexed determination of target Abs in both centralized and point-of-care settings, and proved their diagnostic potential in selected cohorts including healthy subjects and patients diagnosed with SLE, SS, and AD. The results to be discussed in this communication demonstrated the pioneering potential of the bioplatforms as well as the selected biomarker signature for the differential diagnosis of these increasingly prevalent CDs, also establishing the serum cut-off values for each of the Abs targets.

Acknowledgements
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O6. MATRIX METALLOPROTEINASES AND TISSUE INHIBITORS OF METALLOPROTEINASES INFLUENCE IN BREAST CANCER

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Keywords: *mass spectrometry, tissue bioimaging, breast cancer, metalloproteinase, tissue inhibitors of metalloproteinases, immunoassay.*

Breast cancer is the leading cause of cancer death in woman. Although, important progress in breast cancer diagnosis and early treatment have reduced cancer-specific mortality, great efforts are still being made to decrease the rate of recurrence and metastasis. It is now clear that the tumor microenvironment plays an important role in cancer development. The microenvironment is composed by distinct cell types (immune cells, fibroblasts, endothelial cells and adipocytes) and the extracellular matrix (ECM) (proteins, glycoproteins and proteoglycans) and it has been implicated in a wide variety of essential biological processes. Currently, most investigations are focused on functional characterization of ECM components and how they relate to the processes involved in cancer pathogenesis and response to therapy.

Matrix Metalloproteinases (MMPs) are a family of endopeptidases requiring Zn^{2+} for their enzymatic activity which promotes extracellular matrix degradation[1]. Under physiological conditions, MMPs activity is tightly controlled by 4 endogenous tissue inhibitors of metalloproteinases (TIMPs). However, MMPs are upregulated in most human tumor cell lines and their high levels are linked to metastasis. The balance between MMPs and their tissue inhibitors play a crucial role in cancer progression and metastasis[2].

The Matrix metalloproteinase 11 (MMP11) was identified as a highly expressed protein in the stromal cells of breast cancer and has been implicated in cancer progression. This work investigates the prognostic significance of tumoral and stromal MMP11 expression in breast cancer tissues and its role in breast cancer progression. An immunohistochemistry-assisted laser ablation–inductively coupled plasma–mass spectrometry (LA–ICP–MS) method was developed for the quantitative bioimaging of MMP-11 in breast cancer tissues. Results showed that MMP-11 is significantly up-regulated in breast metastatic samples compared to non-metastatic ($P = 0.0077$) and healthy breast tissue ($P = 0.0087$).

Furthermore, it has been demonstrated that Conditioned Media from human Uterine Cervix Stem Cells (CM-hUCESCs) possesses antitumoral effect on the malignant breast cancer line MDA-MB-231[3]. A Semiquantitative Proteomic study by ESI-MS/MS showed the presence of high levels of TIMP-1 and TIMP-2 in the CM-hUCESCs. The anti-tumoral effect of TIMP-1 and TIMP-2 of CM-hUCESCs and TIMPs-depleted CM-hUCESC was investigated on a breast cancer cell line.

Acknowledgements

Sara Escudero-Cernuda thanks the Principality of Asturias for the "Severo Ochoa" grant (PA-21-PF-BP20-067). This work has been funded by the Principality of Asturias through project GRUPIN IDI 2021/000081, the Ministry of Science and Innovation of Spain through project PID2019-109698GB-I00 and by the Instituto de Salud Carlos III (ISCIII) through project PI20/01122 and co-funded by the European Union.

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O7. TETRAZOLE HYBRIDS TARGETING BETA-AMYLOID AND METAL IONS IN ALZHEIMER'S DISEASE

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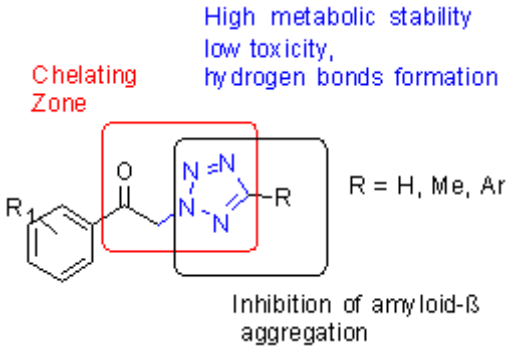
Keywords: *Alzheimer's disease, tetrazole hybrids, metal chelation, beta-amyloid protein, neuroprotection.*

Neurodegenerative disorders are undergoing a worrying increase in incidence, affecting to more than 50 million people worldwide. Among them, Alzheimer's disease (AD) is the most prevalent, representing an 80% of the total dementia cases[1]. According to the "metal ion hypothesis" and "amyloid cascade hypothesis", abnormal accumulation of metal ions in brain increases beta-amyloid ($A\beta_{42}$) aggregation, triggering the neurodegeneration observed in AD[2].



In this work, four tetrazole hybrids have been evaluated as neuroprotective multi-target compounds against AD. They were designed to contain a chelating zone with high metabolic stability, low toxicity and hydrogen bonds formation, and different substituents that could modulate beta-amyloid affinity (**Figure 1**).

***Figure 1**. Structure of tetrazole hybrids*

Therefore, chelation against metal ions (Fe(II), Cu(II) and Zn(II)) has been studied employing UV-Vis spectroscopy and its first derivative. By analysing metal-tetrazol solutions at different ratios (1:1-1:4) and applying chemometric methods, the presence of an interaction and its possible stoichiometry were elucidated. Inhibition of amyloid-aggregation in presence and absence of metals was evaluated by Transmission Electron Microscopy, with the proper measure of fibrils width to give a deeper insight into beta-amyloid morphology. Neuroprotection was confirmed when inhibition of metal-induced amyloid aggregation was observed by tetrazole hybrids, considering them as potential multi-target compounds against AD.

Acknowledgements
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O8. SALIVARY ESTRADIOL AS BIOMARKER FOR THE EVALUATION OF THE MENSTRUAL CYCLE: FACT OR MYTH?

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Keywords: *estradiol, saliva, menstrual cycle, liquid chromatography, mass spectrometry.*

Estrogens are sexual steroid hormones derived from cholesterol involved in different biological processes. In humans, estrogens (mainly 17β-estradiol (E2), but also estrone (E1)) are pivotal molecules involved in growth, nervous system maturation, bone structure, menstrual cycle and pregnancy. Salivary steroid immunoassays are widely used in psychoneuroendocrinological studies of menstrual cycle phase, puberty, and menopause. In spite of manufacturers advertise their assays as suitable, a recent study showed that cycle phase poorly predicted salivary estradiol values using these immunoassays, showing an upward bias compared to expectations from serum[1].

On this basis, this work is focused on the development and validation of an analytical methodology for the determination salivary E2 levels at sub pg/mL levels. The method is based on derivatization by 1,2-dimethyl-1*H*-imidazole-5-sulphonyl chloride (5-DMIS-Cl, a reagent for derivatizing phenols[2]) and analysis by ultra-high performance liquid chromatography coupled to tandem mass spectrometry. Starting with 1 mL of saliva, a first liquid-liquid extraction (LLE) with tert-butyl methyl ether was performed. After derivatization with 5-DMIS-Cl, and a second LLE with n-hexane, organic layer was evaporate and redissolved with 50 µL of water:methanol 1:1. MS/MS data were acquired in selected-reaction monitoring (SRM) applying the recurrent acquisition of the same SRM transition and signal summing for increasing absolute response[3]. The high-specificity of the SRM transition simplified chromatographic separation, which was achieved using a generic C18 analytical column and water/methanol gradient (both with 0.01% formic acid and 1 mM ammonium formate) as mobile phase.

Finally, method was successfully validated following the European Medicines Agency (EMA) at sub pg/mL levels, and applied to authentic female saliva samples at different menstrual cycle phase, in order to check if salivary E2 levels can be considered or not suitable biomarkers for evaluating the menstrual cycle.

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O9. DETERMINATION OF THE EXOGENOUS AND ENDOGENOUS METAL CONTENTS INSIDE CELLS VIA SINGLE-CELL ICP-MASS SPECTROMETRY (SC-ICP-MS): AN OVERVIEW THROUGH CASE STUDIES

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Keywords: *single-cell, ICP-mass spectrometry, cell biology, nanomedicine.*

Cell biology and nanomedicine strongly benefit from having access to information on the metal contents with single cell resolution, rather than at population scale. Metals are intrinsically present in cells to fulfil a wide variety of biochemical functions or are deliberately imported into cells, *e.g.*, in the context of novel cell therapies, relying on the use of nanotechnology. However, very few analytical techniques are capable of quantifying metals at single-cell resolution. Novel technological breakthroughs in ICP-mass spectrometry (ICP-MS) have shown the potential of this technique for high-throughput single-cell analysis[1].

Single-cell ICP-MS (SC-ICP-MS) relies on the one-by-one introduction of cells into the ICP, where they are individually vaporized, the molecules atomized, and the atoms thus obtained ionized. The ion cloud thus formed is then introduced into the mass analyzer, and the transmitted ions finally detected produce signals of very short duration (*ca.*, 0.5 ms). After appropriate calibration, this approach provides information on the absolute amount(s) of the target element(s) in individual cells (*e.g.*, average and median concentration, concentration distribution). SC-ICP-MS shows promising features for the early detection and treatment of various diseases as well as for an evaluation of the efficacy of (chemo-)therapeutic treatments, but this approach is still in a very early phase.

This work will discuss the (bio)analytical challenges still hampering a wider use of the technique and will provide an overview of the application range through different case studies. First, results for the quantification of several endogenous elements and of Pt, present as a result of exposure of various cell types to cisplatin as a Pt-containing chemotherapeutic drug, will be presented, and the differences in Pt uptake between cell types will be linked to chemosensitivity and chemoresistance. Differences in the metal(loid)s contents will also demonstrate the potential of this technique as a “metallo-fingerprinting” tool[2]. Furthermore, the potential changes in the homeostasis of blood cells will be evaluated. Special attention will be paid to QA/QC of the (bio)analytical protocol, while results of a biological consistency test and of serial dilution experiments will be used to further assess the validity and relevance of the method. Finally, a novel methodology for quantifying the number of nanoparticles (NPs) attached to the membrane of an individual cell will be optimized. This approach shows potential for finetuning cell-based therapies, such as NP-mediated photoporation.

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O10. DISTURBANCES IN *MUS MUSCULUS* MICE PLAMA SELENOPROTEOME CAUSED BY THE DICLOFENAC EXPOSURE. ANTAGONISTIC INTERACTION WITH SELENIUM

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Keywords: *diclofenac, selenium, selenoproteome, isotopic dilution and ICP-QQQ-MS.*

The presence of pharmaceutical residues in aquatic ecosystems derived from the extensive use of pharmaceutical drugs in humans, is a problem of growing interest due to the intrinsic biological activity of these compounds. As a general rule, pharmaceutical residues and/or their metabolites are usually detected in the environment at trace levels (ng L^{-1} to µg L^{-1}), below the therapeutic doses used for medical purposes, but even at these low levels, can induce toxic effects[1]. Diclofenac (DCF), a non-steroidal anti-inflammatory used to prevent pain and inflammation, is one of the most commonly detected pharmaceuticals in water and several research has been carried out to demonstrate its long-term effects on living organisms. In this study, we evaluated the impact on *Mus musculus* mice plasma selenoproteome after the exposure to an environmental relevant DCF dose. In addition, in this experiment antagonistic interactions with selenium supplementation (Se) were evaluated because of Se is an essential element for mammals, and the antagonistic interaction of this element against the toxicity of some xenobiotics has been widely studied[2]. To this end, 40 *Mus musculus* mice were used and divided into four groups: control group, group exposed to diclofenac (DCF) (20 mg/Kg bw), group supplemented with Se-enriched feed (0.65 mg/kg) and group exposed to DCF in water and supplemented with Se. A method for the simultaneous speciation of selenoproteins and total selenometabolites has been developed and applied to mice plasma based on separation by two-dimensional liquid chromatography (size exclusion and affinity chromatography) and detection by triple quadrupole inductively coupled plasma mass spectrometry (ICP-QQQ-MS). The method enables simultaneous quantitative analysis of selenoprotein P (SePP), extracellular glutathione peroxidase (eGPx), selenoalbumin (SeAlb) and selenometabolites in serum by species-nonspecific isotopic dilution (SUID)[3]. The results show an increase in the levels of GPx, SeAlb and SePP in the mice supplemented with Se compared to the control group. However, when mice are exposed to DCF, a decrease of semetabolites and SeAlb is observed compared to the control group. Moreover, SeAlb levels are restored to control levels when mice are simultaneously exposed to DCF and supplemented with Se. These results confirm that exposure to DCF and supplementation with Se cause alterations in selenoproteome and highlight the possibilities of analytical metallomic approaches as key tools to elucidate the mode of action of drugs in mammals.

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O11. FAST AND INNOVATIVE MICROEXTRACTION TECHNIQUE µSPEED® COMBINED WITH UHPLC-MS/MS FOR THE MULTICOMPONENT DETERMINATION OF PYRROLIZIDINE ALKALOIDS IN EDIBLE FLOWER INFUSIONS

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Keywords: *pyrrolizidine alkaloids, microextraction, UHPLC-MS/MS, food safety, flower infusions, µSPEed.*

Pyrrolizidine alkaloids (PAs) are natural toxins produced by plants of different families Asteraceae, Fabaceae, Boraginaceae, Orchidaceae, and Apocynaceae. Their intake is mainly associated to liver damage, but they can also produce genotoxic and carcinogenic effects at long-term exposure. Although, we do not directly eat these plants, many recent food alerts have notified high levels of these compounds in a wide variety of food products. Indeed, 15% of these alerts have been indicated in teas and infusions made from plants and flowers, making the occurrence of these toxins a current problem in the food safety field[1]. Accordingly, flower infusions, such as mallow, calendula, and hibiscus, are products increasingly consumed by the population because of their gastrointestinal, relaxing, anti-inflammatory, and expectorant properties[2]. Consequently, due to the potential risk for human health that the continuous and frequent intake of these products may entail, it is of utmost importance to monitor the occurrence of PAs in food by high-throughput analytical procedures. Microextraction techniques have gradually gained attention due to their many advantages over conventional methods (e.g., minimal use of organic solvents, low amount of sample and user-friendly systems). This leads to the development of ecofriendly procedures, which meet the green analytical chemistry principles. Accordingly, this works describes the multicomponent microextraction of 21 PAs set in legislation with the innovative µ-SPEed® technique followed by their analysis by UHPLC-IT-MS/MS in order to propose a sustainable and sensitive analytical methodology to monitor the occurrence of these alkaloids in flower infusions (mallow, calendula and hibiscus). The µ-SPEed® is a solid-phase-based microextraction procedure, which uses small sorbent particles (<3 µm) tightly packed in a disposable needle equipped with a pressure-driven valve to withdraw sample flow in a single direction at high pressure (up to 1600 psi)[3]. The procedure was designed and optimized. The steps were: the conditioning and activation of the C18 cartridge (4 mg) using 2 aspiration-dispense cycles of methanol, followed by 2 cycles of water. Then, the sample loading using 3 cycles, and finally the elution with 1 cycle of methanol. The final procedure just took 1 min per sample, only using 300 µL of organic solvent and 300 µL of sample per extraction. Then, the sample extracts obtained were analyzed by UHPLC-IT-MS/MS in ESI positive mode. The chromatographic separation was performed on a Luna Omega Polar C18 column, and a gradient elution was carried out combining water containing 0.2% formic and 5 mM ammonium acetate with methanol containing 10 mM ammonium acetate. The chromatographic separation of the 21 PAs was achieved within 10 min. The method was properly validated, providing suitable linearity, selectivity, sensitivity (quantification limits 0.3−1 µg/L), overall recoveries (79−97%), and precision (≤17% relative standard deviation). The method was applied to the analysis of 8 flower infusion samples and revealed similar total PA values (23−41 µg/L) and contamination profile among mallow and hibiscus samples (predominance of senecionine-type and heliotrine-type PAs, respectively). Conversely, calendula samples showed more variations (23−113 µg/L), highlighting the occurrence of intermedine *N*-oxide and europine *N*-oxide on them[4].

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O12. NEUROPROTECTIVE POTENTIAL OF BIOACTIVE COMPOUNDS FROM AVOCADO RESIDUES

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Keywords: *avocado by-products, circular economy, polyphenols, in vitro neuroprotection, antioxidant capacity.*

Avocado (*Persea americana*) is a fruit widely consumed fresh or processed, the production of which has increased significantly in recent decades. Its industrial processing generates by-products, namely peels and seeds, which represent up to 30% of the fruit. As a result, the processing industries must deal with the generation of high quantities of waste, as well as the negative effects of waste management. These wastes are often underutilized despite their potential as a renewable, abundant and cheap source of functional molecules with high added value, such as polyphenols, known for their beneficial properties for human health. The development of approaches for the green valorization of these wastes, focused on the production of novel bioactive raw materials, could be an appropriate strategy to mitigate the environmental problems associated with their management, while improving the sustainability and economic competitiveness of the food industry. Hence, residual avocado peels and seeds can be exploited for the development of multifunctional agents against oxidative-related diseases such as Alzheimer's disease (AD), one of the most concerning neurodegenerative disorders worldwide. Abnormal accumulation of metal ions, such as Fe(II), Zn(II) or Cu(II), is one of the pathologies of AD, leading to the formation of beta-amyloid plaques. For this reason, antioxidant and metal chelators are being sought for the development of novel neuroprotective agents and nutraceuticals[1,2].

In this context, this work aimed at the valorization of avocado by-products for obtaining functional and neuroprotective extracts rich in phenolic compounds. A green extraction method was developed and optimized, employing water as solvent, and assessing different techniques (solid-liquid (SLE), ultrasound (USE), and microwave assisted (MAE) extraction). The phenolic composition was determined using spectrophotometric methods and liquid chromatography coupled to mass spectrometry. Furthermore, the neuroprotective potential of the phenolic extract was tested against a set of *in vitro* assays: anti-amyloidogenic activity, DPPH radical scavenging activity, lipid peroxidation inhibition and cytotoxicity reduction assay, employing human neuroblastoma cell line (SH-SY5Y). The results demonstrated that the avocado extracts obtained under the optimal conditions (SLE, 30 min, 60 ^{o}C) were rich in gallic acid, *p*-coumaric acid, *trans*-ferulic acid, resveratrol and kaempferol. Furthermore, the promising *in vitro* neuroprotective and antioxidant activities (IC_{50} = 63 ± 5, $ng \cdot g^{-1}$) suggested that avocado by-products extract could be used as functional ingredient in pharmaceutical and food industry.

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O13. SINGLE-CELL-ICP-MS FOR STUDYING THE ASSOCIATION OF INORGANIC NANOPARTICLES WITH CELLS DERIVED FROM AQUACULTURE SPECIES

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Keywords: *scICP-MS, ammonia-based DRC technology, titanium dioxide nanoparticles, silver nanoparticles, nanoparticles uptake in cells, aquaculture species.*

Because of their outstanding physicochemical properties, inorganic and metal oxide nanoparticles are widely used in many everyday consumer products. The large-scale production and the release of inorganic nanoparticles in the marine environment concerns to the scientific community because of the potential risk of nanoparticles for the environment and for animal and human health. As a result, data are required to assess the environmental risk of these emerging pollutants, mainly data regarding the uptake of inorganic nanoparticles in the biota and its implications at the biological level.

The current research deals with the use of single cell inductively coupled plasma – mass spectrometry (scICP-MS) for the assessment of titanium dioxide nanoparticles (TiO_2 NPs) and silver nanoparticles (Ag NPs) association in cell lines derived from aquaculture species (sea bass, sea bream and clams). The optimization studies have considered the avoidance of high dissolved background, multi-cell peak coincidence, and possible spectral interferences. Optimum operating conditions were found when using a dwell time of 50 µs for Ag NPs and 100 µs for TiO_2 NPs. In addition, the assessment of associated TiO_2 NPs was performed by Dynamic Reaction Cell technology (ammonia flow rate at 0.75 mL min^{-1}) and monitoring the $^{48}Ti(NH)(NH_3)_4$ adduct (m/z ratio of 131). The influence of other parameters such as the number of washing cycles and the cell concentration on the accurate determinations by scICP-MS was also fully investigated. Ultra-sensitivity derived from scICP-MS analysis was demonstrated by reaching limits of detection at attograms per cell level, not provided by other instrumental techniques. The application of the developed methodology to kidney cells extracted from kidney of the sea bass and sea bream, and from gill of clam has shown a great influence of the nanoparticle size distribution (5.0 and 25 nm for TiO_2 NPs and, 15 and 100 nm for Ag NPs), the TiO_2 NPs / Ag NPs exposure concentration (10 and 50 µg mL^{-1} for TiO_2 NPs and 5.0 and 50 µg mL^{-1} for Ag NPs), and the cultured species on the degree of association of nanoparticles to cells. Additional characterization of the NPs-cell interaction by TEM and SEM shows that the NPs not only internalized, but also associated strongly to the cellular membrane. The morphology and ultrastructure of the non-exposed cells observed by TEM indicated that haemocytes are the predominant cells. In addition, TEM images show that TiO_2 NPs are mostly adsorbed on the cellular membrane, while cellular uptake of Ag NPs is observed.

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O14. AUTHENTICATING BEE POLLEN ORIGIN BY USING DIFFERENT ANALYTICAL TECHNIQUES

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Keywords: *amino acids, bee pollen, betaines, bioactive compounds, chemometrics, GC, glucosinolates, HPLC, ICP, minerals, sample treatment, method validation.*

Bee pollen has been used in the human diet for many centuries. Its ever-increasing consumption results from its nutritional value and its health-promoting effects, like those relating to its antioxidant, anti-inflammatory, anticarcinogenic, antibacterial or anti-fungal properties. The nutritional value/quality and health properties of bee pollen are linked to its constituents, which include proteins, amino acids, lipids, carbohydrates, phenolic compounds, vitamins, and minerals, among several other compounds. However, its composition varies greatly according to several factors, like botanical and geographical origins, climatic conditions, the type of soil, or harvesting and processing conditions. This is quite important to prevent one of the main problems currently affecting the commercialization of bee pollen and thus the beekeeping industry, which is the fraudulent practice of adulteration with pollen from other sources/origins, such as, for instance, pine pollen. As may be expected, studying the profile of a particular family of compounds (proteins, amino acids, lipids, phenolic compounds, or minerals) in bee pollen has been proposed to specify/authenticate its origin as well as to evaluate its corresponding nutritional value or health-promoting effect.

Therefore, the main goal of this presentation is to investigate the potential of minerals and three families of bioactive compounds scarcely investigated in this matrix (amino acids, betaines and glucosinolates) as bee pollen markers, by determining with different analytical techniques like high performance liquid chromatography (HPLC), gas chromatography (GC) and inductively coupled plasma (ICP) their respective content in bee pollen samples from experimental apiaries located within the same area (Marchamalo, Guadalajara, Spain); The samples analyzed were collected in three consecutive harvesting periods in the same year (April-May; June; July-August). It should be mentioned that in most cases, new analytical methods which fulfilled some of the principles of green analytical chemistry were proposed, and they were fully validated according with current legislation. Results showed that bee pollen samples can be classified, in most cases, by means of a canonical discriminant analysis (CDA) based on the content of the different compounds, according to the corresponding apiary of origin or the harvesting periods. Therefore, the potential of these compounds as bee pollen markers was demonstrated, and, in addition, new analytical tools to authenticate the origin of bee pollen were provided.

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O15. FLUORESCEIN-GOLD NANOPARTICLES FRET-BASED DETECTION OF miRNA USING NUCLEIC ACID ENZYMES AS AMPLIFICATION TOOL

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Keywords: *MNAzymes, FRET, microRNA, AuNPs, signal amplification.*

MicroRNAs (miRNAs) are noncoding, highly conserved, single-stranded and endogenous RNAs made of 18-25 nucleotides which are considered as an emerging class of biomarkers. A growing number of scientific publications have reported a correlation between their presence with pathological conditions, such as inflammation diseases, cancer, tuberculosis, etc[1]. Therefore, it is essential to develop appropriate bioanalytical methods to carry out an accurate and highly sensitive detection of this novel biomarkers. In this sense, detection of a miRNA can be based on the hybridization of the target strand with complementary oligonucleotides. However, despite such hybridization is highly specific, the concentration levels of miRNA detected are rather high for clinical, food or environmental applications.

The present work shows a methodology that takes advantage of a novel signal amplification strategy using Multicomponent Nucleic Acid Enzymes (MNAzyme) based on fluorescence energy transfer (FRET) between fluorescein and gold nanoparticles (AuNPs)[2,3].

For this purpose we have selected the organic dye FAM (derived from fluorescein) as energy donor, and AuNPs as energy acceptor. Additionally, the MNAzymes employed are capable of specific hybridizing the analyte, giving rise to a conformation that catalyzes the cleavage of an RNA substrate.
Two different oligonucleotides functionalized one with AuNPs, and the other with a fluorophore, being both partially complementary to the substrate, have been designed.

In absence of the target, the substrate remains intact, keeping the donor an acceptor in close proximity, and giving rise to a high FRET efficiency (i.e. low fluorescence emission). However, in presence of the miRNA, the MNAzyme is activated, breaking the substrate in two parts. This cleavage produces an increase in the distance between donor and acceptor, causing an increase in the fluorescence emission as consequence of a lower FRET efficiency. Results obtained for the determination of a miRNA in raw milk samples will be shown in this communication.

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Comunicaciones orales-jóvenes

OJ1. TRACKING LESSER-KNOWN ALLERGENIC PROTEINS AND CARBOHYDRATES WITH ELECTROCHEMICAL BIOPLATFORMS

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Keywords: *food allergy, soybean, red meat, electrochemical immunoplatform.*

The prevalence of food allergy has been steadily increasing in recent decades. Current dietary trends are altering eating habits, expanding the list of potential allergens to be closely monitored. For example, soy has recently been introduced in Western countries to produce meat and milk analogues in vegan diets, and since 1929, soy-based milk has been used as a substitute for children sensitive to cow's milk. Unfortunately, it was only 14 years later that sensitization to soy-based formulas was first reported. Nowadays, soy is among the most common human food allergens, known as the "big eight". Despite this, it is widely used as an ingredient in meat, dairy, and bakery products, cheese analogues, desserts, soups, etc[1][2]. However, food allergy does not exclude the main rival of soy-based or vegan diets, and meat allergy must also be taken into account. In this regard, allergens from red meat, have been poorly explored and historically considered rare. Today, red meat allergy is not uncommon and, surprisingly, almost all cases are related to specific IgE to galactose-α-1,3-galactose (α-Gal), a non-primate mammalian oligosaccharide. Recently, it has been reported that sensitization to α-Gal could be triggered by tick bites and that α-Gal specific IgEs adversely affects cancer therapy, demonstrating the need for research on the link between nutrition and health[3].

Although several analytical methods are available to detect food allergens, there is still an urgent demand to fine-tunning tools capable of scanning allergens of different omic level in a fast, simple, affordable, and point-of-care manner. In this context, modern electrochemical biosensing methods are showing adequate versatility, flexibility, and sensitivity to successfully respond to this demand[2]. Being aware of all this, our most recent research has addressed the development of two electrochemical immunoplatforms for the dual detection of the main soy allergenic protein targets (glycinin and β-conglycinin), and of the main mammalian oligosaccharide (α-Gal) associated with red meat allergy. Both methodologies developed involve the use of magnetic particles (MPs) and specific antibodies, tracers and immunoassay formats for each target, performing amperometric detection on dual or single disposable carbon chips using the H_2O_2/hydroquinone system. The dual bioplatform developed to trace soybean exhibits good selectivity and sensitivity providing detection limits of 0.03 and 0.02 ng mL^{-1} for β-conglycinin and glycinin, respectively, and pioneering detection, using the dual checkpoint, of cookie extract samples incurred with as little as 0.5 μg Kg^{-1} of soybean meal in as little as 1.5 h[2]. The bioplatform for α-Gal demonstrates its detection in the fM range in a single step and is currently addressing the analysis of red meat. These bioplatforms are the first described to date for the detection of these antigens and their flexibility to detect allergenic targets, regardless of their omic level (protein or carbohydrate) and origin (plant or animal), together with their ease of use and compatibility for multiplexed on-the-spot determinations, should also be highlighted. These unique characteristics invite to consider them promising tools for ensuring food safety and labeling regulation, for further advancing emerging allergenic targets research, and for assisting precision nutrition.

Acknowledgements
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OJ2. NANOPARTICLE-BASED ENHACEMENT IN NANOCHANNEL SENSORS FOR MONITORING ENZYMATIC CLEAVAGE: APPLICATION TO MMP-9 DETECTION

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Keywords: *nanochannels, nanoparticles, electrochemical, MMP-9.*

The impact of nanotechnology and materials science in the advancement of biomedicine has notably increased over the last few decades. In particular, outstanding properties of nanomaterials have converted them in powerful tools for sensing applications. Among the different nanomaterials used in biosensing, nanopores/nanochannels have stand out in the last years due to their high applicability in the biosensing field[1]. Nanoporous alumina membranes stand out from these materials, due to their easy functionalization, capacity for mass production and biocompatibility[2].

In this context, in this work we propose a novel methodology using nanoparticles as carriers of an enzymatic substrate immobilized inside the nanochannels, with the aim of amplifying the blocking produced and, consequently, improving the efficiency of the enzyme determination through the enzymatic cleavage action (**Figure 1**). Streptavidin modified polystyrene nanoparticles (PSNP) are proposed as carrier agents, applied to the detection of matrix-metalloproteinase 9 (MMP-9), a potential biomarker overexpressed in different diseases.

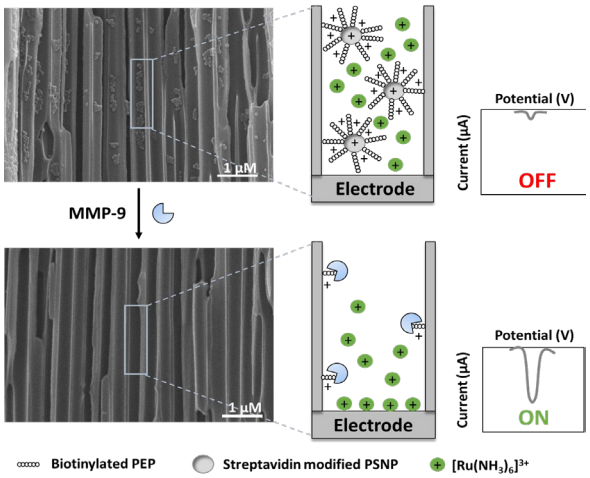

Figure 1. *Schematic methodology (not in scale) of the biosensing system based on the blockage of nanochannels by the PSNP/PEP conjugate and further unblockage by enzymatic cleavage of MMP-9. SEM (cross-sectional view) images correspond to the inner walls of the nanoporous alumina membranes with PSNP/PEP immobilized (up) and after the reaction with MMP-9 and a washing step (down).*

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OJ3. RAPID SCREENING OF NOVEL ANTIMICROBIAL COMPOUNDS WITH A NANOPOROUS-BASED ELECTROCHEMICAL PLATFORM

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Keywords: *antimicrobials, nanochannels, electrochemistry, biosensors.*

The over-use of antimicrobial treatments has led to the emergence of resistant superbugs unable to be killed by the compounds out in the market[1]. Developing novel antimicrobial compounds is then a need. But unfortunately, the high cost of development and the long-time required of gold-standard antimicrobial susceptibility tests (AST), has made industries leave the antibiotic innovation race. The development of reproducible and high-throughput AST methods, as electrochemical sensors, stands out as a solution to solve this challenge[2].

In this work, we have developed an electrochemical sensing platform based on the use of nanoporous alumina membranes (AAO) for the evaluation of novel antimicrobial compounds. Thanks to the low unspecific absorption and biocompatibility of AAO membranes, they are suitable platform for the *in vivo* culture of bacteria[3].

AAO membranes are modified in this work for the selective capturing of pyocyanin and enterotoxin B, two virulence factors secreted by *Pseudomonas aeruginosa* and *Staphylococcus aureus* respectively, the predominant bacteria in chronic wounds. Once the virulence factors are immobilized inside the nanochannel, they block the passage of a redox indicator, reducing the current recorded. In the presence of an effective antimicrobial compound, virulence factor release is reduced, thus unblocking the nanochannel, and increasing the current recorded.

Compared to traditional AST methods, the developed sensor allows the differentiation between the bactericidal and bacteriostatic effects of a novel antimicrobial compound and helps to easily determine the minimum inhibitory concentration required.

The electrochemical platform developed in this work is not only useful for the screening of novel antimicrobial compounds, but also opens the path to a more personalized medicine, allowing an *on-demand* antibiotic administration.

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OJ4. USE OF GOLD NANOPARTICLES TO DEVELOP A SMARTPHONE-BASED POINT-OF-CARE BIOSENSOR FOR PROSTATE SPECIFIC ANTIGEN ULTRASENSITIVE QUANTIFICATION

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Keywords: *biosensor, immunoassay, point-of-care, nanotechnology, smartphone.*

Nowadays, one of the major goals in medicine is the fast identification and quantification of biomarkers, which are key pieces for the study of biological processes. Many of them are found in very low levels of concentration in complex biological medias, hence there is a high demand for ultrasensitive and reliable approaches to detect these biomarkers. Among them, Prostate Specific Antigen (PSA) is a widely studied biomarker of prostate cancer that can be also secreted by breast and ovary. This makes PSA a potential biomarker for early diagnosis of breast or ovary cancer. However, it can be found that PSA concentrations in women serum are on the order of pg/mL, being these levels often lower than the Limit of Detection of many clinical methods[1]. That is why there is an urgent need for developing methodologies able to perform ultrasensitive detection and monitor disease biomarkers at such low concentration levels, focusing efforts on easy to use and low-cost procedures in order to develop point-of-care systems.

Fur such purpose, the first step was to improve immunoassay sensitivity. This was achieved by tagging an anti-PSA antibody with gold nanoparticles (AuNPs) which act as catalytic tags, to carry out a size-enhancement through selective silver electrodeposition once the immunoassay is done[2]. After controlled silver electrodeposition on the surface of AuNPs, a size-enhancement of the nanoparticles from nm to µm was observed. The immunoassay was carried out on ITO (Indium Tin Oxide) coated PET (Polyethylene terephthalate), which allows both electrochemical and optical detections.

Due to such enhancement, the quantification of biomarkers can be carried out in different ways, through electrochemical and optical detection. The proposed strategies exhibited limit detections (LOD) of 91 and 37 fg/mL for electrochemical and optical detection respectively, achieving required sensitivity to detect biomarkers at this concentration levels for early diagnosis and monitor eventual recurrence after clinical treatments. Moreover, to assess the high specificity of the immunoassay, serum of healthy woman was analyzed showing excellent results and non-interfering effects of other proteins or serum compounds.

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OJ5. USING COMPLEMENTARY ANALYTICAL TECHNIQUES FOR THE STUDY OF PROTEIN CORONA FORMATION ONTO PtNPs IN DIFFERENT BIOLOGICAL MEDIA

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Keywords: *PtNPs, biological media, protein corona, analytical techniques.*

In recent years, the evolution of nanotechnology and development of new nanomaterials has resulted in the application of nanoparticles (NPs) in different fields due to their excellent physicochemical properties. In biomedicine, platinum NPs (PtNPs) have been studied and promising results were obtained as potential treatment or diagnostic tools[1]. When NPs are dispersed in a biological media, different macromolecules, including proteins, are adsorbed onto their surface. This results in a new biological identity for NP known as protein corona (PC)[2]. This structure is described to have two parts depending on the affinity NP-protein. In the hard corona the proteins that form part of it establish strong interactions with the NP. Otherwise, if the affinity between them is lower, there is a continuous exchange between the proteins that gives rise to a more dynamic structure known as soft corona. The formation of PC modifies the physico-chemical properties of NPs, affecting their behavior, fate, and toxicological profile. Therefore, it is of great importance to understand the process of PC formation on these NPs.

The PC formation is a complex process and complementary analytical techniques should be used, as they can provide different and reliable information. For this purpose, non-spectroscopic (dynamic light scattering (DLS)), spectroscopic (absorption of ultraviolet-visible radiation (UV-vis) and fluorescence emission), and separation techniques (asymmetric flow field flow fractionation (AF4) and sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE)) have been employed. The formation of PC onto PtNPs (30 and 50 nm) have been evaluated in phosphate buffer with bovine serum albumin (BSA) and two serum types (fetal bovine and human), at two concentration levels (1 or 10%) and at different incubation times (0, 1, 24 h). As expected, PC formation increased hydrodynamic diameter of both PtNPs for all media used, finding significant differences between hard and soft corona and between media. Likewise, Z-potential of PtNPs evolved from negative to positive values being slightly higher in the case of human serum. Results revealed that UV-vis absorption spectrum of PC complex is a strong predictor of NP-protein interaction and the concentration of proteins retained on the NP surface. By fluorescence emission studies, it was possible to observe the quenching process carried out by PtNPs in the native emission of BSA. Besides, the use of hyphenated AF4-ICP-MS or AF4-UV-vis has also allowed to differentiate between hard and soft corona and confirm the isolation of the NP-protein complexes from free proteins. Finally, the SDS-PAGE technique was used to study the composition of the biocorona in hard corona after incubation in serums. Different band profiles were obtained for each of them. Nevertheless, in both serums the majority band was found around 66 kDa (molecular weight of albumin) and the intensity of this band increases over time. The use of complementary techniques has been proven to be beneficial to achieve deep knowledge of the processes that PtNPs may undergo in different biological media and further studies are required.

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OJ6. ENZYMATIC SYNTHESIS OF NANOMATERIALS AS INDICATORS IN OPTICAL BIOSENSORS

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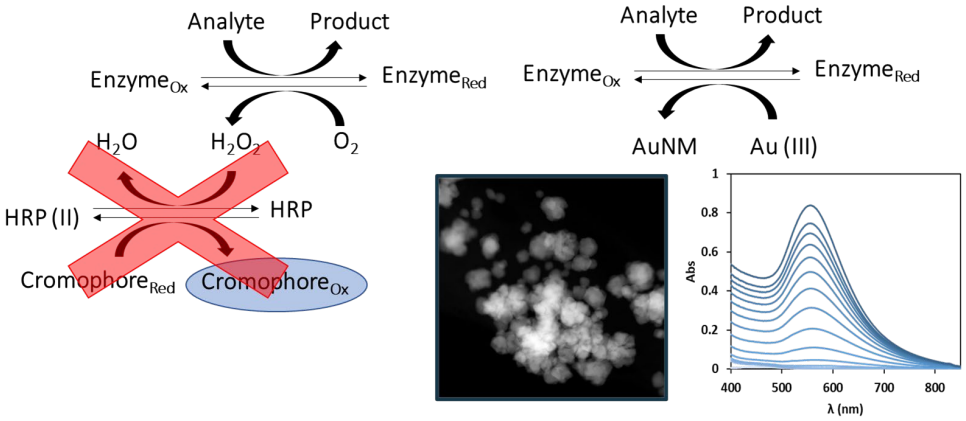
Keywords: *enzymatic biosensors, nanomaterials, colorimetric.*

Optical enzymatic biosensors are mainly based on an oxidase type-enzymatic reaction coupled to an indicating reaction. This indicating reaction usually involves the oxidation of a dye by H_2O_2 catalyzed by peroxidase (HRP). However, several problems frequently appear in these reactions such as the instability of the dye, the dye/HRP lateral reactions and the lack of specificity of HRP. Some of these drawbacks can be overcome with an alternative colorimetric method based on the in-situ formation of metallic nanomaterial, avoiding the indicating reaction and whose optical properties can be related to the concentration of the analyte.

This work presents the results with Au (III) as a precursor of the gold nanoparticles or nanoclusters, which optical properties (SPR or Fluorescence, respectively) have been related to the concentration of biogenic amines (such as cadaverine, tyramine[1] or putrescine) or biological parameters (such as glucose[2]). The different parameters (pH, enzyme and gold concentration, temperature and type of buffer) were studied in order to optimize the reaction and to understand the mechanism. Also, in order to improve the sensitivity, other metals (Cu, Ag, Pt, Pd) and its combination with Au (III) were tested.



The studies have also focused on understanding the reaction, trying to develop a kinetic model which justify the formation mechanism of the corresponding nanomaterials and its relationship with the concentration of analyte. To demonstrate the role of the enzyme in the formation of the nanomaterials, this methodology has been applied in enzymatic reactions with flavoenzymes (i.e. Glucose oxidase or Putrescine Oxidase) or amino-oxidases with cupper as cofactor (i.e. Tyramine oxidase or Diamine oxidase).

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OJ7. CHARACTERIZATION OF EXTRACELLULAR VESICLES FROM HUMAN UTERINE CERVIX FOR ITS IMPLEMENTATION AS ALTERNATIVE THERAPY

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Keywords: *extracellular vesicles, exosomes, particle characterization, standardization.*

Mesenchymal Stem Cell's (MSC) Secretome is the set of all biologically active molecules (Growth Factors, Chemokines, Cytokines and Extracellular Vesicles (EVs)…) produced by the cells and secreted into the Extracellular Matrix and has a considerable potential in regenerative medicine as a Cell-Free therapy. EVs, including Exosomes, Microvesicles and Apoptotic Bodies, are important mediators of intercellular communication in multiple biological and pathological processes. Among EVs, Exosomes are attracting great attention due to their ability to reproduce the therapeutic effects of MSCs. Exosomes are lipid bilayer particles (30–150 nm diameter) bearing numerous biological molecules including lipids, proteins, RNAs. Despite the advantages of Cell-Free treatments, most MSC Secretome-based therapies have not been implemented because the complexity of the secreted bioactive factors is not completely understood.

Secretome from human Uterine Cervix Mesenchymal Stem Cells (hUCESCs) has been proved to have antitumoral, antifungal and corneal regeneration capacity among other effects[1,2]. For the implementation of new therapies based on hUCESCs-EVs a great knowledge of its composition is required. Currently, EVs studies have some limitations such as lack of standardization, a usual insufficient analytical characterization in the bibliography and a terminology inconsistency[3,4].

In this work, hUCESCs EVs has been isolated by differential ultracentrifugation. The combination of complementary methods capable of detecting, characterizing, and quantifying extracellular vesicles were optimized: size and morphology by Transmission Electron Microscopy, size distribution and particle concentration by Nanoparticle Tracking Analysis, total protein content by a Bicinchoninic Acid Assay and purity by Size Exclusion Chromatography. Moreover, a method has been developed for Exosomes identification using specific biomarkers (CD9 and CD81) and their membrane integrity by Flow Cytometry. An alternative method for particle concentration determination is proposed. These methodologies were applied to the exhaustive characterization of hUCESCs-EVs obtained under different *in vitro* culture conditions and to commercial Exosome Standards.

Current challenges limiting characterization of EVs are discussed: the lack of standardized EVs reference materials and standardized isolation and characterization protocols which could allow researchers to validate EVs samples.

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OJ8. DETERMINATION OF CHIRAL THYROID HORMONES IN HUMAN MILK BY SOLID-PHASE MICROEXTRACTION AND HOLLOW FIBER LIQUID PHASE MICROEXTRACTION FOLLOWED BY AN ANALYTICAL MULTIPLATAFORM COMBINING IMMS, UHPLC-QTOF AND ICP-QQQ-MS

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Keywords: *thyroid hormones, human milk, solid-phase extraction, hollow fiber, chiral, column-switching, IMMS, ICP-MS, UHPLC-QTOF.*

Thyroid hormones (TH) are the only compounds that contain iodine with biological activity and, for this reason, this element is essential for mammals. These hormones intervene in many developmental and metabolic processes related to the central nervous system and foetal neurodevelopment[1]. The major form of TH in the blood is thyroxine (T4) that is converted in the most active triiodothyronine (T3) by deiodinases (selenoproteins). T4 exists as two enantiomeric forms, while T3 has two positional isomers. The determination of the individual TH is essential because they have different biological, pharmacological and therapeutic effects[2]. TH are very difficult to separate due to the variability of chemical properties such as structure, solubility, pKa and chirality. Human milk (HM) is a complex and constantly changing mixture of endogenous and exogenous substances including TH with important health relevant impact in the infant[3].

In this work, we developed a new analytical method based on column switching ultra-high performance liquid chromatography (UHPLC) combining chiral and reversed stationary phases for the separation of 8 TH in HM including chiral and positional isomers that has not been previously reported in this biofluid. Moreover, we have combined three analytical multiplatform including UHPLC coupled to quadrupole time of flight (QTOF) for the unequivocal determination of TH using tandem mass spectrometry, HPLC coupled to inductively coupled plasma triple quadrupole mass spectrometry (ICP-QQQ-MS) for the sensitive determination of TH by heteroatom-tag proteomics using iodide as the “tag” and ion mobility mass spectrometry (IMMS) for collision cross section (CCS) determination. For sample treatment we have combined liquid-liquid extraction (LLE) followed by solid phase extraction (SPE) and hollow-fibre liquid phase microextraction (HF-LPME) in three phase mode. The most important advantages of HF-LPME are among others the simplicity of operation and required equipment[5], robustness, low cost, disposable character (avoiding memory effects) and high enrichment factors. Although nowadays HF-LPME is a very promising analytical technique, under our knowledge, there is not previous works reporting its application for the extraction of TH from HM.

The developed multiplatform analytical method is sensitive, simple, reliable, reproducible and allows determining 8 TH in HM at natural levels. The method has been satisfactorily applied to 160 HM samples including iodine deficiency and control lactating women, in which we have also determined metabolomic and microbiota profiles. Clinical variables were also recordered. The final objective is to determine potential links between human milk microbiota, TH and metabolites related to maternal iodine deficiency. Our results opens further research to control the presence of TH in human milk and warrants further research due to the potential implications for infant health.

Acknowledgements
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OJ9. STUDY OF TITANIUM DIOXIDE NANOPARTICLES IN FISH, MUSSELS, AND SEAWEED SAMPLES FROM THE ATLANTIC AREA

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Keywords: *salmon, sea bass, sea bream, mussel, Alaria esculenta, SP-ICP-MS.*

Due to their high use, TiO_2NPs are released into the environment, where they interact with marine organisms. Mussels and algae can be considered bioindicators of contamination, and fish tend to accumulate contaminants through the food chain. Then, these marine organisms can be considered potential targets for nanoparticle bioaccumulation. Thus, for this research work, several fish, seaweed, and mussel samples were collected in strategic regions from the Atlantic area to monitor the presence of total titanium content and TiO_2NPs.

For total titanium determination, the samples were submitted to microwave-assisted acid digestion with HNO_3 69% (v/v), H_2O_2 33% (v/v), and ultrapure water, before their analysis by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS).

On the other hand, for TiO_2NPs determination, samples were submitted to alkaline and enzymatic extractions before Single-Particle-ICP-MS (SP-ICP-MS) analysis. Replicates of 1.0000 g of homogenized and crushed seaweed were mixed with 7.5 mL of 2.5% (v/v) of TMAH and submitted to sonication in an ultrasonic bath for 2 hours. Masses of 1.0000 g of homogenized fish and mollusk samples were mixed with 7.5 mL of 8.0: 8.0 g L^{-1} of a pancreatin: lipase solution prepared in 0.2M NaH_2PO_4/0.2M NaOH (pH = 7.4) buffer. The extracts were incubated overnight at 37 ºC and 200 rpm for titanium nanoparticles extraction. Both kinds of extracts were filtered with filter disks of regenerated cellulose (5 µm), diluted several times with 1% (v/v) glycerol, and analyzed by SP-ICP-MS[1,2].

Finally, the methodologies were applied to the total titanium and TiO_2NPs number concentration and size distribution determination in several wild *Salmo*, *Dicentrarchus labrax*, *Sparus aurata*, *Mytillus edulis*, and *Alaria esculenta* specimens from Ireland, France, and the United Kingdom, by ICP-MS and SP-ICP-MS, respectively.

Acknowledgements

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OJ10. GAS CHROMATOGRAPHY-COMBUSTION-MASS SPECTROMETRY, A POWERFUL TOOL TO SIMULTANEOUS UNIVERSAL AND QUANTITATIVE ELEMENTAL DETECTION

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Keywords: *mass spectrometry, gas Chromatography, selective detector.*

Mass spectrometry has established as one of the most universal and useful detector in gas chromatography due to its capacity to provide universal detection together with structural identification and compound-specific detection (SIM mode). Unfortunately, the structural-dependent signal makes it essential to resort to species-specific standards for quantification. Additionally, identification of specific families of compounds (N, S or O) in complex matrix is very limited being mandatory the use of selective detectors (SCD and NCD) for their detection and quantification.

The GC-combustion-MS system presented in 2010[1] allows to provide generic universal quantification of organic compounds while maintaining the structural elucidation capabilities of MS. A combustion interface installed between the column and the MS enabled to convert all the organic compounds (containing C) eluting from the column into CO_2 quantitatively. Notably, the most important heteroatoms potentially present in organic molecules (H, N, S) could be detected as well by their corresponding volatile oxide species (H_2O, NO and SO_2)[2]. Therefore, it can be considered as the first instrumental system that enables online and simultaneously ultrasensitive elemental quantification, both universal (C, H) and family-specific (N, S), of individual organic compounds eluting from the GC column.

The novel set-up consisted of a ceramic tube (400x3x0.5 mm) with 2 Pt wires located inside of a combustion oven and a two position 6-way valve. The online combustion process takes places inside the ceramic tube reactor at temperatures ranging from 850 to 1150 ºC after mixing the elute of the column with an online flow of He:O_2. The conversion of all the eluted compounds to the same volatile species (CO_2, H_2O, NO and SO_2) prior to MS detection enables complete compound-independent quantification opening the door to the use of simple, cheap and certified generic standards. LODs in ppb level or below are within reach for each target elements (C, S, N, H). The robustness of the approach was validated by the analysis of complex real samples.

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OJ11. HIGH-PERFORMANCE VOLTAMETRIC SENSOR BASED ON CHITOSAN/ɣ-CYCLODEXTRIN-GRAPHENE QUANTUM DOTS FOR GLOBAL ESTIMATION OF FLUOROQUINOLONES IN COMMERCIAL FOOD DAILY PRODUCTS FROM ANIMAL SOURCE

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Keywords: *voltammetric approach, bionanocomposite assembly, graphenic nanostructure, animal-source foods, host–guest receptors.*

A new electrochemical platform based on screen printed carbon electrodes (SPCE), modified with a composite nanomaterial assembled from gamma cyclodextrins functionalized graphene quantum dots and chitosan (δCDs-GQDs/CHI), has been reported for the first time to evaluate the global amount of fluoroquinolones (FQs). Synthetized nanomaterial exhibits an extraordinary electrochemical behavior towards fluoroquinolones oxidation due to the excellent conductivity of GQDs incorporated on chitosan film. Additionally, δCDs became an excellent recognition element allowing a selective size-based discrimination of FQs over other drugs[1].

For the design of the electrochemical sensing platform, GQDs-COOH were synthesized from uric acid by acidic chemical treatment[2]. Succinic acid was used as linker for the GQDs functionalization with different CDs (α-CDs, β-CDs and δ-CDs), which were later evaluated over the performance of the electrochemical sensing system. Nanostructural characterization of the developed composite was also performed. Thus, GQDs size and morphology were assessed by HR-TEM (4.3 ± 0.5 nm) and hydrodynamic diameter (11 nm) was obtained by DLS. Z potential measurements confirms the negative surface charge of the graphene species due to partial substitution of carboxyl groups (-11 mV); in consequence, chitosan was selected as cationic polymer for a suitable anchoring to the working electrode through electrostatic charges. XRD spectra were recorded to confirm graphenic structures. 1730 cm^{-1} peak of IR spectra, which is attributed to C═O stretching from the ester formation in the coupling of CDs corroborate the correct functionalization of GQDs. Electrochemical properties of the sensing system were assessed by CV using potassium ferricyanide as redox probe, showing an increase on electronic transfer rate (k^o) and electroactive area (A) in presence of δCDs-GQDs. Electrochemical oxidation mechanism of the redox analyte was studied on four representative quinolones, obtaining in all cases the same number of e- (2) and H^+ (2) involved in their oxidation process.

Analytical performance features were investigated by means of a mixture of four FQs selected as representative of the electrochemical behavior of the whole family. Very good results were obtained in terms of linear range (4 – 250 µM), lower detection limit (LOD = 1.2 µM), together with excellent repeatability, reproducibility and selectivity. Developed sensor allowed the determination of FQs global contain in broths and dairy samples at three concentration levels (150, 75 and 37.5 µM) with recoveries in the 90-110% range.

Acknowledgements

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OJ12. NANO-LIQUID CHROMATOGRAPHY AFTER DISPERSIVE LIQUID-LIQUID MICROEXTRACTION FOR THE SIMULTANEOUS CHIRAL ANALYSIS OF DRUGS IN WATER SAMPLES

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Keywords: *nano-liquid chromatography, dispersive liquid-liquid microextraction, enantiomeric separation, drug, water samples.*

Nano-liquid chromatography (nano-LC) is a powerful analytical technique to perform fast studies on separative analyses and to explore the full potential of novel stationary phases mainly due to short analysis and equilibration times. Dispersive liquid-liquid microextraction (DLLME) is a sample preparation technique that complies with the features of quick, easy, cheap, reliable, safe, and environmentally friendly, and enables to achieve satisfactory results in the extraction of different pharmaceuticals from environmental waters[1]. For a fully sustainable sample preparation, safe extraction solvents alternative to chlorinated ones should be used in DLLME, such as the recently introduced isoamyl acetate, which gave satisfactory recoveries of 12 pesticides from urine samples[2].

In this research work, the chiral separation of ten drugs from different families was carried out by nano-LC using a silica with immobilized amylose tris(3-chloro-5-methylphenylcarbamate) column. The recognition capability of this chiral stationary phase was investigated by evaluating different mobile phase compositions and changing the organic solvent/water content, salts/buffers concentration, and pH values. The best enantiomeric separations of the analyzed drugs were obtained when a mobile phase of methanol/water at pH 10.0 was employed and using ammonium carbonate. Subsequently, the effect of the buffer concentration in the mobile phase (ammonium carbonate concentration from 10 to 75 mM) was evaluated to achieve the simultaneous separation of as many drug enantiomers as possible. Finally, a DLLME treatment based on the use of isoamyl acetate as green solvent was carried out to increase the sensitivity of the developed nano-LC methodology *via* preconcentration of four chiral drugs (alprenolol, mianserin, tolperisone, and temazepam) considered as emerging drugs contaminants in environmental waters. Thus, tap water and Tiber River water were analyzed by the DLLME-nano-LC method developed obtaining good recoveries (> 70%) for three of the four chiral drugs (recovery for tolperisone was ~40%).

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OJ13. QUANTIFICATION OF ULTRA-TRACE GRAPHENE OXIDE IN REAL WATER SAMPLES BY SERS

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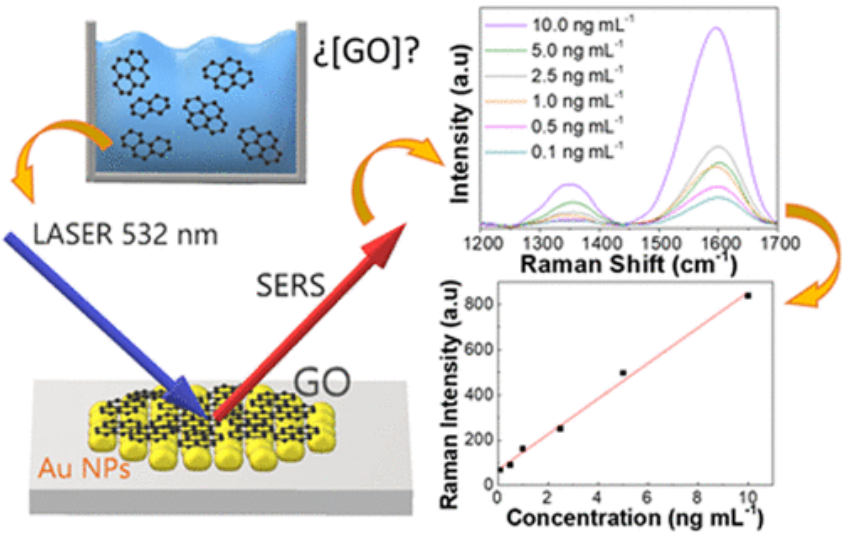
Keywords: *SERS, Graphene oxide, ultra-trace, quantification, gold nanoparticles.*

The use of graphene oxide (GO) increases in many products due to its vast number of excellent properties. However, this extensive use in real-world applications has increased their potential release into the environment. To evaluate their possible health and ecological risks, there is a need for analytical methods that can quantify these materials at very low concentrations in environmental media such as water. The selected analytical technique must allow differentiation between carbon nanomaterials and other carbon compounds present in real water samples. For this reason, Raman spectroscopy is applied as a perfect option, specifically its most innovative variant, surface-enhanced Raman scattering (SERS). This technique allows to detect and quantify GO with high selectivity and sensitivity. This method is able to detect GO in the concentration range of 0.1–10.0 ppb. Using SERS, in this work was achieved a quantification method of GO at trace levels[1], 0.1 ng mL^{-1}, which is lower than the predicted concentrations for graphene in effluent water reported to date. And the recoveries obtained ranged from 95.66% to 100.47%. This methodology has been successfully applied to samples of real filtered water with GO filters and release of GO was excluded[2].



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Pósters

P1. ELECTROCHEMICAL MOLECULARLY IMPRINTED POLYMER SENSOR FOR SELECTIVE DETERMINATION OF EMERGING CONTAMINANTS IN WATER

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Keywords: *electroanalysis, electrochemical sensors, molecularly imprinted polymer (MIPs), emerging contaminants, antidepressant drug.*

The increasing presence of pollutants in environmental waters is an alarming problem, not only because of their harmful effects on the environment, but also because of their risk to human health. Studies carried out to assess water quality usually focus on priority pollutants such as heavy metals, hydrocarbons, microbial toxins, and additives. However, recent research reveals the presence of a multitude of organic compounds (such as pharmaceuticals or personal care products) that significantly affect water quality, even in small concentrations (micropollutants). These emerging contaminants (ECs) are difficult to remove in wastewater treatment plants (WWTPs), so a large part of them and/or their metabolites may enter the effluent.

Therefore, the analysis of these ECs is essential, but this is difficult due to the great variety of contaminating substances. Facing this analytical challenge, electrochemical sensing based on molecularly imprinted polymers (MIPs) has become an interesting strategy for environmental monitoring. Thanks to their superior chemical and physical stability, low-cost production, high selectivity and fast response, MIPs combined with miniaturized electrochemical transducers offer the possibility to detect target analytes on-site[1].

In this context, this work presents the preliminary results obtained in the development of a simple, low-cost and miniaturized electrochemical molecularly imprinted polymer sensor for the determination of an antidepressant drug. The selective MIP sensor was constructed via direct imprinting of the antidepressant drug onto the surface of a commercial screen-printed carbon electrode, through electropolymerization of a solution containing monomer and drug. This sensor is easy to prepare and use, and can be integrated with small portable readers for on-field analysis.

Acknowledgements
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P2. PENCIL-DRAWN ELECTROCHEMICAL ENZYMATIC SENSOR FOR TYRAMINE DETERMINATION IN FISH

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Keywords: *electrochemical biosensor, paper-based electrochemical cell, pencil-drawn electrode, tyramine, tyrosinase, food quality.*

Food safety is a growing concern since the rise of the world's population and globalization has led to the increase of needed foodstuff and their transport around the globe. Furthermore, every year almost 1 in 10 people in the world falls ill from consuming contaminated food. One of the sources of foodborne illnesses are biogenic amines (BAs). Among these, tyramine and histamine are considered the most toxic. Although they are normally present in fish and fermented foods, their concentration rapidly increases when the foodstuff is not correctly stored. The consumption of high levels of these BAs results in health problems for consumers and, consequently, in economic and legal problems for food suppliers.

Therefore, the development of analytical tools that are able to quantify these BAs in a simple, rapid, and decentralized way will contribute to guarantee food quality and safety along the entire production chain. With the aim of accessing remote places and low-income countries, these analytical devices should not only be robust, but also cheap and easy to fabricate and transport. In this context, paper-based electroanalytical platforms form an excellent basis for the development of low-cost and disposable point-of-need biosensors, since paper is a cheap, light, and widely available material, and small-sized electrochemical cells with suitable analytical features can be easily designed on paper.

In this work, a paper-based electrochemical cell was developed and used as the transducer of a tyrosinase-based biosensor for tyramine quantification. The cell consisted of a pencil-drawn working electrode, combined with metallic pins (provided by a standard commercial connector) as reference and counter electrodes[1]. The composition of the used pencil, as well as the enzyme concentration and the detection potential (for chronoamperometric readout), were assessed to achieve optimal electroanalytical features. This simple biosensor showed a useful dynamic range, as well as high reproducibility, and suitable selectivity. Moreover, it was successfully applied to recovery tests in fish samples such as tuna and salmon.

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P3. TRANSITION METAL DICHALCOGENIDE-BASED JANUS MICROMOTORS FOR ON-THE-FLY *SALMONELLA* DETECTION

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Keywords: *Janus, affinity peptides, micromotors, fluorescence, endotoxins.*

The utilization of self-propelled micromotors in (bio-)sensing assays has led to a fundamentally new approach where their continuous movement around the sample and the mixing associated effect, due to the generated microbubbles tail, greatly enhances the target-receptor contacts and hence the binding efficiency and sensitivity of the assay. In this context, Janus polymeric micromotors, named after the Roman god Janus, are the subject of intense research in the current analytical scientific scenario due to their biocompatible properties and the possibility of functionalization and incorporation of nanomaterials.

Janus micromotors encapsulating transition metal dichalcogenides (TMDs) and modified with a rhodamine (RhO)-labeled affinity peptide are used here for *Salmonella enterica* endotoxin detection. As shown in **Figure 1** the OFF–ON strategy relies on the specific binding of the peptide with the TMDs to induce fluorescence quenching (OFF state); which is next recovered due to selectively binding to the endotoxin (ON state). The increase in the fluorescence of the micromotors can be quantified as a function of the concentration of endotoxin in the sample. The developed strategy was applied to the determination of *Salmonella enterica* serovar Typhimurium endotoxin with high sensitivity (limits of detection (LODs) of 2.0 μg/mL using MoS_2, and 1.2 μg/mL using WS_2), with quantitative recoveries (ranging from 93.7 ± 4.6% to 94.3 ± 6.6%) in bacteria cultures in just 5 min. No fluorescence recovery is observed in the presence of endotoxins with a similar structure, illustrating the high selectivity of the protocol, even against endotoxins of *Salmonella enterica* serovar Enteritidis with great similarity in its structure, demonstrating the high bacterial specificity of the developed method. These results revealed the analytical potential of the reported strategy in multiplexed assays using different receptors or in the design of portable detection devices.

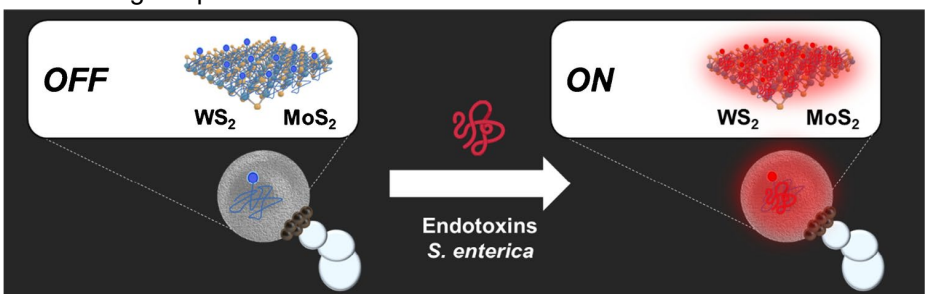

Figure 1. *Salmonella enterica* endotoxin detection strategy using affinity peptide-modified Janus micromotors.

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P4. BIOELECTROCHEMICAL PLATFORM COMBINING A LECTIN-MIMICKING APTAMER AND AN ANTIBODY FOR THE DETECTION OF SERUM AMYLOID PROTEIN (SAP)

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Keywords: *aptamer, liquid biopsy, electrochemical biosensors, pancreatic cancer.*

In our research group, we have selected an aptamer capable of distinguishing between the natural glycosylated form and the recombinant form (without glycans) of PSA protein by using SELEX methodology[1]. Through selective PSA deglycosylation assays, it was concluded that this aptamer recognizes external sugars, mainly sialic acids and galactoses, but not the peptide region. For this reason, this aptamer could be used as a generic receptor for the detection of glycans from other proteins, thus emulating the natural receptors, namely lectins, but with higher affinity and with the advantages of stability and synthesis typical of an artificial receptor[2].

Thus motivated, we developed a sandwich-type electrochemical biosensor for the detection of serum amyloid P protein (SAP), a potential biomarker of pancreatic cancer, combining a specific antibody for this glycoprotein and the lectin-mimicking aptamer as capture and detection receptors, respectively. Two oriented antibody immobilization approaches onto self-assembled monolayers built to gold electrodes were evaluated comparatively. The resulting biosensor platform can detect the SAP protein at levels of ng/mL with a reproducibility value of less than 10%, both in aqueous buffer and in serum.

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P5. INVASIVENESS-FREE MANAGEMENT OF PANCREATIC CANCER VIA AN IMMUNOELECTROCHEMICAL PLATFORM WITH CLINICAL PROSPECTIVE CAPABILITIES

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Keywords: *immunoplatform, amperometry, CA19-9, pancreatic cancer, serum samples.*

The legendary fight against oncological diseases is supported, among other major pillars, by the discovery, evaluation, and judicious clinical practice of tumor markers. In this sense and despite the valuable information provided by a wide variety of all omic degree biomarkers, unfortunately, only a few of them are currently approved by the Food and Drug Administration (FDA) to be clinically exploited in cancer management[1]. With a particular focus on one of the most lethal and aggressive forms of human cancers, pancreatic ductal adenocarcinoma (PDAC), carbohydrate antigen 19-9 (CA19-9) ranks as, among all the revealed pancreatic tumor-related biomarkers to date, the sole validated monitoring marker[2] for evidencing suspected PDAC in an invasiveness-free manner.

Concerned about the incidence, as well as by the pessimistic statistics associated with PDAC, we modestly contribute to this struggle by sharing a simple but highly competitive electrochemical transduction mode-based immunoplatform, uplifted in a sandwich-type configuration onto micromagnetic pearls, for the rapid and sensitive serological quantification of the FDA-approved pancreatic marker CA19-9[3]. Importantly, the proposed immunosensing approach skillfully blends, in a groundbreaking mode, the unique benefits of magnetic microsupports (MBs) and screen-printed electrode transducers (SPEs) for the amperometric interrogation of CA19-9 target marker, previously sandwiched between specific capture and HRP-tagged detector immunoglobulins, in just one hour and by exhibiting attractive enough characteristics from the analytical, cost-effective, and point-of-care application points of view compared to the conventionally available methodologies. The proposed bioplatform may be considered as a firmly useful electrochemical bio-tool complementing the more popular gold-standard methods for the prompt and reliable diagnosis of PDAC. Under optimal assay conditions, our immunoplatform permits to accurately determine the presence of the targeted biomarker in a range of concentration levels spanning 5 to 500 U mL^{-1} CA19-9, with a limit of detection (LOD) of 1.5 U mL^{-1}, remarkable storage stability, good reproducibility, and distinguished selectivity.

Full exploitation of all the above tempting attributes exposed by the developed electrochemical platform was conclusively confirmed by testing a medium-sized cohort of diluted serum samples from 6 and 16 healthy subjects and patients diagnosed with PDAC, respectively. Achieved findings solidly revealed the unchallenged potential of our proposed immunosystem to positively identify PDAC patients discriminating between healthy and PDAC-diagnosed subjects, thus making our simple but functional methodology to be positioned as an advanced electroanalytical tool with demonstrated real biomedical applications, with the hope of contributing to the reliable diagnosis and follow-up of one of the top-three human cancers with the lowest expected overall survival rate.

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P6. BIOELECTROANALYTICAL TOOLS AT THE FOREFRONT TO IDENTIFY CANCER AGGRESSIVENESS

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Keywords: *electrochemical bioplatform, colorectal cancer, angiogenesis, metastasis, human endostatin.*

Angiogenesis is one of the fundamental hallmarks of cancer pathophysiology. Angiogenesis is a physiological process consisting of the formation of new blood vessels from pre-existing ones thatplays a key role in tumour progression and metastasis. This is why research into this process and the identification of relevant targets associated with its development is considered a top priority in an era in which there is a clear need to move progressively towards a sustainable precision medicine that is accessible to all, improves the survival and quality of life of patients and ensures a rational use of resources. In this context, endostatin is one of the most studied peptides with inhibitory effect on angiogenesis located in the extracellular matrix. Some studies have shown that high levels of endostatin are related to the development of aggressive cancers and therefore with worse prognosis[1].

To further explore the role of this molecular target in angiogenesis, our research group has recently worked on the development of a biotool for its determination that offers competitive advantages over conventional methodologies in terms of simplicity, speed and point-of-care application. The bioplatform combines the benefits of using magnetic microparticles, sandwich immunoassay formats and amperometric transduction on disposable carbon electrodes.

In our hands and working under exhaustively optimized conditions, the innovative electroanalytical tool developed offers sensitivity (LOD of 34.1 pg mL^{-1} for the amperometric determination of standards) and selectivity suitable for its foray into the clinical oncology area. With this objective, the precise determination of the target biomarker in relevant clinical samples -lysates and secretomes of colorectal cancer (CRC) cells with different metastatic potential, and plasma and tissue samples from patients with CRC in different stages- has also been faced with satisfactory results. According to our experimental results, the developed bioplatform allows quantitative determinations to be carried out with precision, requiring minimal pretreatments and sample amounts and in just 75 min, features that demonstrate its attractiveness compared to the usual ELISA or immunoblotting technologies.

Acknowledgements
The financial support of PID2019-103899RB-I00 (Spanish Ministerio de Ciencia e Innovación) Research Projects, PI17CIII/00045 and PI20CIII/00019 Grants from the AES-ISCIII Program co-founded by FEDER funds and the TRANSNANOAVANSENS-CM Program from the Comunidad de Madrid (Grant S2018/NMT-4349) are gratefully acknowledged. M.G-A. acknowledges the postdoctoral contract Margarita Salas for the requalification of the Spanish University System. A.M-C. was supported by a FPU predoctoral contract supported by the Spanish Ministerio de Educación, Cultura y Deporte.

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P7. SPECTROELECTROCHEMISTRY AT ULTRAMICROELECTRODES

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Keywords: *spectroelectrochemistry, UV-vis absorption, ultramicroelectrodes, antioxidants.*

Spectroelectrochemistry (SEC) is a powerful technique that provides a better understanding of interfacial phenomena.[1] Although SEC experiments are well established for macroelectrodes (disk electrodes, screen-printed electrodes, among others), access to ultramicroelectrodes (UMEs) has not yet been achieved, due to the difficulties of arranging the entire SEC setup in a limited space. UMEs present great advantages for chemical analysis due to their small size, such as low capacitive currents, high mass transport due to radial diffusion or the capability to work in low concentrations of supporting electrolyte. UV-Vis absorption SEC is particularly interesting since it provides molecular information related to the species involved in the electrode process, being possible to follow the kinetics of reactants and products during the electrochemical reaction.[1] Therefore, being able to combine UV-Vis absorption SEC with UMEs opens new gates in the study of electrochemical processes. However, this combination requires a miniaturization of the optical system due to the micrometer size of the electrode. In this work, micrometer diameter optical fibers together with high resolution positioning systems have been combined to obtain SEC measurements in UMEs. As can be seen in **Figure 1**, the optical signal (evolution of absorbance at 260 nm) reproduces the electrochemical signal of the UME in this sampled system.

An additional advantage of the measurements with UMEs is the possibility of using a scanning electrochemical microscopy (SECM) setup, being able to obtain a triple response: one obtained from substrate, a second one corresponding to the UME and, finally, the optical signal which is obtained by interrogating the solution confined between the substrate and the UME. In this way, the different SECM working modes can be used to obtain a full understanding of the electrochemical process, opening new gates to study complex electrochemical systems.

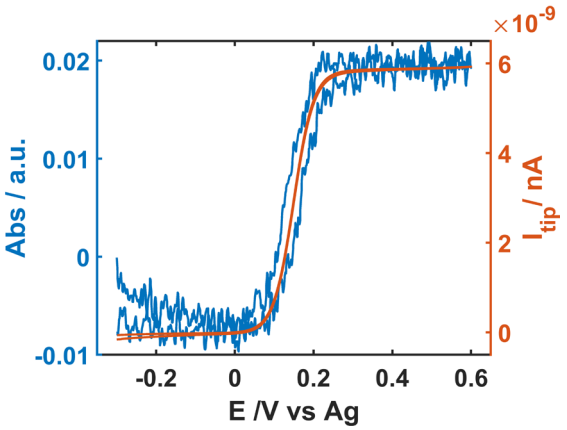

***Figure 1**. Comparison of the voltammetric response for a Pt UME and the absorbance at 260 nm during the oxidation of Ferrocenemethanol 5 mM in KCl 0.1 M.*

Acknowledgements

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P8. LABEL-FREE DIRECT NUCLEIC ACID HYBRIDIZATION DETECTION WITH SURFACE MICROPATTERNED BIOFUNCIONALIZED HYDROGELS

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Keywords: *hydrogels, surface micropattern, probe immobilization, photoclick reaction, diffraction, label-free.*

Surface diffractive micropatterns made of functionalized hydrogels have demonstrated promising performance as traducers in label-free optical sensing[1]. However, there are very few examples using bioreceptors, mainly antibodies, to achieve biosensing, with their use for nucleic acid hybridizations not being reported. In this communication, the optimization of surface diffractive micropatterns based on Acrylamide/Propargyl acrylate (AM/PA) hydrogels is described. The hydrogel is used, simultaneously, as a matrix for the pattern fabrication and for the biofunctionalization with single-strand thiolated oligonucleotides, immobilized via thiol-yne photoclick chemistry[2]. The thiolated probes act as biorecognition elements of complementary DNA. Two different polymerization strategies, thermal and photochemical; and two different immobilization approaches, during or after the hydrogel synthesis; were tested. The PA comonomer was shown to significantly contribute to the immobilization of the probes. The hydrogel polymerized by thermal curing and functionalized after the polymerization yielded the best properties for the selective detection of targets as observed by fluorescence imaging. In addition, it has good consistency to allow the fabrication of the surface micropatterned structure. Therefore, this hydrogel was assessed for the label-free detection of complementary DNA strands by monitoring the diffraction efficiency changes after hybridization. The hydrogel showed a selective response to the hybridization reaching a limit of detection of 2.47 µM. With the aim of improving the durability, storage, and stability of the biofunctionalized micropatterned hydrogel, it was lyophilized and, after rehydration, tested again for label-free DNA biosensing. The results showed that the hydrogels retained their properties while improving their stability. The label-free biosensing system here described could significantly contribute to the development of direct and accurate analysis as it is cheap, stable, easy to fabricate, and works without the need for further reagents.

Acknowledgements

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P9. ENZYME-CARBON NANODOT BIOCONJUGATES AS FLUORESCENT LABELS TO DETECT POLYPHENOLS

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Keywords: *polyphenol determination, carbon nanodots, bioconjugate, laccase enzyme, fluorescent label.*

A direct and simple fluorescent assay for the total polyphenol determination based on the bioconjugate formed between the laccase enzyme (TvL from *Trametes versicolor*) and carbon nanodots (CD) is developed. One of the most used reactions for the determination of phenols is based on the enzymatic reaction of their oxidation to quinones. In this work, CD has been biofunctionalized with TvL (TvL-CD) and employed as a fluorescent label to follow the enzymatic reaction. The bioconjugate was formed and characterized by spectroscopy and microscopy. The reaction between the bioconjugate and a phenolic compound such as gallic acid (GA) was followed by monitoring the fluorescence bioconjugate decrease due to the quenching effect of the quinones generated in the enzymatic reaction. These studies confirm that bioconjugation does not inhibit the enzymatic activity and the fluorescence decrease during the enzymatic reaction is mainly due to an electron transfer process. Based on these results, a new method for the quantitative determination of polyphenols measured as GA concentration is developed. The method has been successfully applied to the direct determination of total polyphenols, measured as GA concentration, in wine, juice, and rice leaf extracts. Results compare well with those obtained from the standard Folin-Ciocalteu method, being the developed method more selective.

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P10. CALCIUM MONOFLUORIDE DIATOMIC MOLECULE GENERATION IN GAS PHASE USING LIBS TECHNIQUE FOR ITS DETECTION AND DETERMINATION

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Keywords: *calcium, CaF molecule, molecular spectrometry, LIBS.*

Laser-induced breakdown spectroscopy (LIBS) is a laser-based analytical technique that explores the emission of a plasma (using a spectrometer) generated through a high-energy laser shot. Briefly, a modulated laser beam (usually between femtoseconds – nanoseconds) is focused onto a (solid) sample. The matrix of the sample absorbs the energy from the laser, leading to its breakdown and, further, to the generation of a plasma, self-sustained with the absorption of the laser energy by the free electrons from the matrix. Obviously, the content (matter) of the plasma is a mixture of the sample particles that were ablated and of the surrounding atmosphere. These particles are excited (some of them even ionized) due to the plasma high temperature, and finally, the emission from the species present in the gas phase is collected with a spectrometer.

LIBS is an elemental analysis technique that predominantly detects ionic and elemental emissions, however, there are studies that evaluate the determination of diatomic molecules targeting one of the atoms as analyte[1]. Such a molecule can be generated if the precursors are found in the matrix or, else by adding the molecule-forming agent (*e.g.*, by mixing the solid sample with a salt containing this molecule-forming agent). Both strategies are usually applied aiming at the determination of non-metals (for which attaining atomic information is nontrivial), or to get access to isotopic information by the isotopic shift phenomena, converting the LIBS technique into laser ablation molecular isotopic spectrometry (LAMIS)[1].

There are other potential approaches that can be deployed in order to generate the target molecule in gas phase. For instance, the determination of fluorine in mouthwash samples was carried out by nebulizing such samples over a calcium carbonate substrate and using the laser to generate the CaF diatomic molecule[2]. In this work, we propose an alternative method for molecule generation, consisting in the reaction of our analyte (calcium) with the molecule-forming reagent (fluorine as CH_3F) in the gas phase, using a commercial LIBS instrument J200 (Applied Spectra, USA).

Moreover, the main goal of this work is to obtain isotopic information evaluating the isotopic shift of the emission of both ^{40}CaF and ^{44}CaF species at 583 nm, which theoretically is 292.3 pm, corresponding to 4 to 5 detection pixels for our instrumental resolution. This molecule generation approach will be evaluated in detail.

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P11. NEXT STEPS IN THE CHARACTERIZATION OF MICROPLATICS VIA ICP-MASS SPECTROMETRY OPERATED IN SINGLE-EVENT MODE

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Keywords: *ICP-MS, single-event, SP-ICP-MS, microplastics, laser ablation.*

The occurrence and potential effect of microplastics (MPs) in the environment, food and human health is a matter of increasing concern. However, up to date, there is no universal technique that could provide a full characterization of MPs, and therefore, the combination of different analytical techniques is required. As a result, there is an urgent need to develop novel analytical methodologies for their detection, quantification and characterization.

ICP-mass spectrometry (ICP-MS) is considered as one of the most powerful techniques for (ultra-)trace elemental analysis. *A priori*, the technique was not considered to be appropriated for carbon monitoring due to its high ionization efficiency and high background coming from ubiquitous carbon species, such as atmospheric CO_2. However, ICP-MS operated in single-event mode shows promising features for the analysis of small carbon-based microparticles. This approach relies on the one-by-one introduction of discrete entities into the ICP-MS and can simultaneously provide information on the chemical composition, size and size distribution, particle mass concentration and particle number density, as already demonstrated for metallic engineered nanoparticles[1]. In 2020, a pioneering work carried out by the authors of this abstract demonstrated for the first time ever that ICP-MS operated in single-event mode can be used as a suitable approach for the characterization of MPs (1 and 2.5 µm polystyrene microspheres) by relying on the carbon monitoring[2].

This presentation will address the next steps in the characterization of MPs via single-event ICP-MS. Attention will be paid to a broader size range and to the characterization of different polymer types. The measurement of various hetero-elements will be evaluated as a potential multi-element fingerprinting tool for the identification of the MP type. Furthermore, the use of laser ablation (LA) as a means of sample introduction will be evaluated. The different methods developed will also be used for monitoring potential exposure to real-life MPs in the context of food safety.

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P12. UPTAKE, TRANSFORMATION AND SPACIAL DISTRIBUTION OF SELENIUM NANOPARTICLES IN GERMINATING RICE SEEDS

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Keywords: *SeNPs, rice seedlings, ICP-MS, single particle, laser ablation.*

Selenium (Se) is an essential element for humans and a beneficial nutrient for plants. However, about 1 billion people worldwide suffer from a Se-deficient diet. Selenium nanoparticles (SeNPs) are attracting increasing attention due to their nutritional benefits, lower toxicity and greater bioavailability compared to other chemical forms of Se. Rice is a staple food for more than half of the world's population, and thus, rice biofortification *via* SeNPs exposure may be a viable alternative to increase Se content in rice and to reduce Se deficiency. For studying NP-plant interactions, the use of adequate analytical techniques is mandatory for obtaining reliable information about the NP uptake mechanisms, the potential bio-transformations, and the spatial distribution of such NPs in plant tissues, since this information would help for understanding the potential pathways for human exposure. However, the characterization of NPs incorporated by plants remains a challenging task, as there are no well-established extraction procedures and measurement protocols. In this study, SeNPs have been synthesized by a chemical method[1,2] and characterized by transmission electron microscopy (TEM) and single-particle inductively coupled plasma mass spectrometry (spICP-MS)[3]. Then, germinating rice seeds have been exposed to the previously synthesized SeNPs, or to sodium selenite (Na_2SeO_3) at different concentrations. A multimethod approach has been used for total Se quantification, SeNPs detection and characterization, as well as for the distribution of Se in plant tissues. The total Se concentration has been determined by ICP-MS following acid digestion. To understand the uptake mechanisms and biotransformation of SeNPs in rice seeds, an enzymatic digestion has been used to extract intact SeNPs for subsequent characterization by spICP-MS. Finally, laser ablation ICP-MS (LA-ICP-MS) has been used to investigate the spatial distribution of Se in radicle and plumule of germinating rice seeds. The results of the present study will contribute to a better understanding of the interactions between NP–plant interaction.

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P13. DEVELOPMENT OF A FLUORIMETRIC SENSOR BASED ON THE USE OF CARBON NANOMATERIALS FOR THE ANALYTICAL CONTROL OF ONCOLOGICAL DRUGS

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Keywords: *graphene quantum dots, Imatinib, kinase inhibitors, chronic myeloid leukemia, acute lymphoblastic leukemia.*

Tyrosine kinase are a set of enzymes belonging to the group of protein kinases, which catalyze the transfer of the phosphate group in the form of ATP to a tyrosine residue in a protein. A tyrosine kinase inhibitor is a type of enzyme inhibitor that specifically blocks the action of one or more protein kinases. Imatinib is the first member of a new class of drugs, which act by specifically inhibiting the enzyme tyrosine kinase which occurs as a particular feature of a type of cancer cell. It is used for diseases such as chronic myeloid leukaemia, gastrointernal stromal tumors, acute lymphoblastic leukaemia and other malignant pathologies. Depending on the type of protein molecule or gene fragment, drugs can be targeted at the molecular or cellular scale. These drugs can bind to cancer sites specifically for tumor cell death or by blocking cell division in the G1 phase.

The carbon nanomaterials used in this research are graphene quantum dots (GQD). This carbon-based material has special characteristics and exceptional properties derived from its nanosize (less than 100 nm), such as low toxicity, stable photoluminescence, chemical stability and pronounced quantum confinement effect. Owing to their properties, the GQDs have been used as fluorescent sensors for detection of many species such as glucose, microRNAs, iron ions and pH, dopamine, ascorbic acid, Cu^{+2}, Al^{+3} and D-penicillamine[1].

In this work, GQDs are used as a quenching based fluorescent nanoprobe for the control and determination of imatinib in different biological samples. GQDs were synthesized from uric acid (UA) by acidic chemical treatment. In this case, 0.5 g of UA were combined with 1 mL of concentrated sulfuric acid and allowed to react under continuous stirring at 200 °C for 1 h. The synthesis product was later neutralized by adding NaOH. After that, it was filtered with a 0.45 µm nylon filter. The yellow synthesis product was stored in absence of light at room temperature[2].

Optical properties of the so synthesized GQD were assessed by UV-Vis and fluorescence. GQDs display and absorption band 260 nm (typically attributed to π-π* transitions of the aromatic C=C sp^2 domains) and 352 nm (may be attributed to n-π* transitions of C=O bond). The excitation and emission appear at 356 nm and 447 nm, respectively. It was experimentally observed that the interaction between the fluorescent nanoprobe and analyte lead to at decrease in the fluorescent emission intensity. Currently, in order to carry out the sensor design several parameters (GQD concentration, imatinib concentration, incubation time and pH) are now under study. In the studies carried out at a pH of 7.25 and a concentration level of 50 ppm on GQD, two linear ranges of response we observed (form 0 to 2 ppm and from 4 to 20 ppm), showing more sensibility for the first range.

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P14. NON-INVASIVE NANOMOF-BASED SENSOR FOR DETECTION OF BIOGENIC AMINES IN FOOD SAFETY

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Keywords: *food safety, luminiscence, nanoparticles.*

One of the main concerns of consumers and health authorities are related with food safety. According to World Health Organization, more than 200 diseases are transmitted by food. It highlights the importance of establishing quality controls to avoid or minimize food crisis. With the key principle of preventing and tackling food crisis, a proposal can be to monitor food safety parameters using non-invasive and real time sensors.

In this regard, we can focus on shelf life extension of fresh products, which a degradation in terms of microbiological and the physicochemical properties can be different during storage and commercialization depending on the product nature. Some compounds, which appear because of the metabolic activities of microorganisms in food, are Biogenic Amines (BA). This BA can be present in all types of foods with high protein or free amino acid content, such as fish, meat or wines, their presence in non-fermented foods is usually undesirable and it is related to microbial spoilage. Putrescine and cadaverine are two of the most common BA, their presence in high concentrations can produce severe toxicological effects in humans[1]. The best method for the detection of BA in food could be a naked eye sensor incorporated in the packaging[1].

These non-invasive sensors can be developed by using the properties of nanoparticles such as NanoMOFs (metal organic frameworks). These MOFs are crystalline nanoparticles composed of ions of a metal or a cluster held in a three-dimensional structure connected via organic ligands. Their porous structure, large surface area, modifiability and luminescent properties have raised the interest in designing sensing platforms based on these MOFs[2].

Herein, we propose with this research work the use of nanoMOFS as colorimetric and/or luminescent indicators of BA presence in fresh food samples. For this purpose, PVC and Emerald MOF membranes were designed and exposed to different BA. Results showed that, exposure of these membranes to different BA modifies colorimetric and luminescent properties of Emerald nanoMOF, and those changes can be observed visually as a qualitative tool for BA presence confirmation. For a quantitative analysis, able to verify the compliance with special legislative requirements laboratory instrumentation (fluorescence) is needed.

Acknowledgements
Funding: Spanish Ministry of Science and Innovation PID2020-117282RB-I00 and MCI-20-PID2019-109698GB-I00. Principado de Asturias Regional Government co-financed by the European Union through the European Regional Development Fund GRUPIN IDI/2021/000081.

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P15. METALLOMICS AND HETEROATOM-TAGGED PROTEOMICS OF LUNG CANCER AND CHRONIC OBSTRUCTIVE PULMONARY DISEASE HUMAN SERUM REVEAL A CRITICAL ROLE OF METALS AND SELENOPROTEINS

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Keywords: *elements, selenoproteins, lung cancer, chronic obstructive pulmonary disease, mass spectrometry, isotopic dilution.*

Lung cancer (LC) is the main responsible of cancer deaths worldwide, while chronic obstructive pulmonary disease (COPD) is the foremost cause among all those that increase the risk of LC[1]. Thus, their joint study can provide new contributions to delve into the pathology of LC. The relevance of elements is essential as about one-third of human proteins need their presence to develop their function and their impairments have been suggested as both, a cause, due to the exposure to high levels, and as an effect of LC onset and progression that led to unbalanced levels. Thus, elements could be linked to cancer progression[2].

An observational study on 191 human serum samples was conducted including COPD patients with varying severity from mild to very severe symptoms, as well as COPD patients who developed LC during the follow-up, LC patients and healthy controls. We combined targeted metallomic analysis to determine 18 elements including: V, Al, As, Mn, Co, Cu, Zn, Cd, Se, W, Mo, Sb, Pb, Tl, Cr, Mg, Ni and U, determined by inductively coupled plasma mass spectrometry (ICP-MS), and heteroatom-tagged proteomics for the selenoproteins in human serum, namely: glutathione peroxidase (GPx), selenoprotein P (SELENOP) and selenoalbumin (SeAlb) by size-exclusion and affinity chromatography coupled to ICP-MS. We have used isotopic dilution for absolute quantification.

COPD severity and LC have a significant impact on the human serum elemental composition and selenoproteins profile. COPD severity reduced the concentrations of As, Cd and Tl and increased Mn and Sb compared to healthy control samples, while LC increased Al, As, Mn and Pb. The human serum selenoproteome was also altered with increased concentrations of GPx and SeAlb in LC and SeAlb in COPD. Other studied subgroups showed also altered metallome and selenoproteome. The data were analysed with appropriate statistical tests.

Apart from unveiling novel COPD and oncogenic potential biomarkers that could be used for early diagnosis, our data provides unique insights about the impact of these diseases in the elemental and selenoproteins profile that may open in a future a novel opportunity of therapeutic targeting and supplementation strategies.

Acknowledgements
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P16. EXPLORING NIR SPECTROSCOPY FOR THE DETERMINATION OF WATER AS ADULTERANT IN GASOLINE AND DIESEL

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Keywords: *NIR spectroscopy, water, gasoline, diesel.*

The adulteration of gasoline and diesel with water is an unethical fraudulent practice that might be increasing as a result of the rising of fuel prices. A small quantity of water is added to gasoline or diesel in such a way that the composition is just not 100% gasoline or diesel but it is sold as if it were[1].

The fundamental chemical vibrations of water molecule are highly active in infrared spectroscopy, and their overtones and combination bands are equally active in Near Infrared (NIR) spectroscopy. In addition, those vibrations produce specific spectroscopic bands that enable the selective detection of water[2].

In this work, gasoline-water and diesel-water mixtures at different proportions from 0 to 10% of water content were purposely prepared and analyzed by NIR spectroscopy directly using the liquid sample analysis compartment. Gasoline and diesel were purchased from a local petrol station. Samples were prepared by quintuplicate and each sample was analyzed five times.

The NIR characteristic bands of water, gasoline and diesel were comprehensively discussed and vibrationally assigned to the corresponding chemical molecular vibrations. Afterwards, a principal component analysis (PCA) was performed for gasoline-water samples as well as for diesel-water samples, in such a way that some groupings were visually observed in the scores PCA plot that seemed to be related to the water content. Because of this evidence, a partial least squares regression (PLS-R) was performed for gasoline-water and diesel-water samples, obtaining good results for both the cross-validation model and the prediction training set.

In conclusion, results have demonstrated that NIR spectroscopy is a useful method to detect the presence of water in diesel and gasoline samples, though further research increasing the number of samples and the number of brands and types of gasoline and diesel needs to be accomplished in order to create a reliable and applicable methodology to real case samples.

Acknowledgements
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P17. A FIRST APPROACH IN FLUORESCENCE MEASUREMENTS USING SMARTPHONES AND DIGITAL IMAGE

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Keywords: *fluorescence, smartphone, digital image, MSLA 3D printing.*

Smartphone-based analysis using digital imaging (DIC) has gained great relevance. Most applications are based on absorbance or reflectance measurements. More recently, fluorescence has also started to be implemented in this type of devices. One of the most interesting aspects is that UV can be used as an excitation source, minimising the signal of the blank when fluorescence is measured as color coordinates (RGB, HSV, CIE Lab).

However, the fluorescence values obtained (measured as color) are affected by a number of variables such as: background signal, light source intensity, smartphone sensor, etc. These variables mean that fluorescence measurements are not comparable between devices, lead to systematic errors and hinder the full potential of the smartphone for this type of measurement. The research group has developed a device (through the use of 3D printing technology MSLA -Masked Stereolithography Apparatus-) a fully functional, compact, small-sized and easy to transport device, that allows fluorescence measurements to be taken and standardised measurements with potentially any smartphone.

One of the advantages of using this technology for fluorescence measurements is that the amount of light captured can be adjusted by modifying the photographic parameters, such as the ISO and shutter speed of the smartphone camera, allowing determinations to be made in different concentration ranges.

This device is now being tested for the enzymatic determination of tyramine and/or histamine. Both analytes are enzymatically oxidised by O_2 catalysed by tyramine oxidase. This reaction is coupled to an indicator reaction (HRP/Amplex Red) which produces a fluorescent product. The reagents (enzymes and dyes) have been implemented on self-developed cellulose strips to which the sample solution is added and the fluorescence is measured as color coordinates with the smartphone.

Acknowledgement
Financial support for this project was provided by the Ministry of Science and Innovation and by the European Union (Project PDC2021-121224-I00 funded by MCIN/AEI /10.13039/501100011033 and by the European Union Next GenerationEU/PRTR) as well as by the Diputación General de Aragón- Fondo Europeo de Desarrollo Regional (DGA-FEDER, E25_20R, N&SB).

P18. SINGLE PARTICLE ICP-MS AS AN INTERESTING ANALYTICAL APPROACH FOR THE CHARACTERIZATION OF PLATINUM NANOPARTICLES IN CELL CULTURE MEDIA

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Keywords: *platinum nanoparticles, single particle inductively coupled plasma mass spectrometry, cell culture media.*

In recent years, platinum nanoparticles (PtNPs) have attracted great interest in different fields due to their exceptional physicochemical properties[1]. Their increasing use in many biomedical or biotechnological applications has been accompanied by a growing concern about their safety and impact on human health[2]. Thus, the comprehension of the interactions between metallic NPs (such as PtNPs) and biological media or cells should be addressed for the assessment of their toxicity and potential risks. For this purpose, analytical methodologies enabling the correct study of these nanomaterials in complex matrices are needed. In this context, single particle inductively coupled plasma mass spectrometry (SP-ICP-MS) stands out as a new and powerful alternative to conventional techniques due to its ability to provide simultaneous information on their concentration, composition, and size distribution. Up to the moment, this analytical tool has been mainly employed to study PtNPs in environmental samples but its application to biological samples has been scarcely explored.

Therefore, in this study the applicability of a new methodology based on SP-ICP-MS for the determination of PtNPs in complex biological samples has been evaluated using a cell culture medium commonly utilized in *in vitro* toxicological assays (Dulbecco's Modified Eagle Medium (DMEM) (without and with a supplementation with antibiotics, or antibiotics and fetal bovine serum (10 %)) as case of study. Optimal data acquisition and processing conditions of SP-ICP-MS analysis were 5 ms as dwell time, 40,000 data points for acquisition, the application of a deconvolution algorithm for data treatment, and the use of a well characterized 30 nm PtNP solution for the transport efficiency estimation. The effect over PtNPs concentration and size of different reagents typically used in the literature to reduce potential matrix effects and improve the sensitivity such as cysteine and hydrochloric acid or a mixture or both has also been checked. There were no major changes in the core size under any of the studied conditions, which could be related to the potential formation of a bio entity conferring stability to PtNPs, and preventing the core from clustering processes. In addition, no remarkable differences were observed in terms of particle concentration under the different tested conditions where quantitative recoveries were reached in all cases.

Hence, the method based on a SP-ICP-MS analysis proposed in this work has been proven to be efficient to characterize and quantify PtNPs in complex biological matrices and, consequently, could be useful for toxicological assays and fate studies.

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P19. GENERATION OF NANOPARTICLES BY REACTION WITH ENZYMATIC COFACTORS AND THEIR USE IN REACTIONS WITH DEHYDROGENASE ENZYMES: DETERMINATION OF TROPANE ALKALOIDS

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Keywords: *tropane alkaloids, tropine, tropinone reductase I, NAD, NADH, gold nanoparticles.*

Noble metal nanoparticles are rapidly increasing in popularity as colorimetric reagents, due to plasmonic band sensitivity. Most of the strategies used are based on modifying the initial color of the nanoparticles by increasing or decreasing their diameter during the enzymatic reaction. Recently, the research group where this study is being carried out has developed a new indicator strategy that is based on the formation of gold nanoparticles during the enzymatic reaction[1]. This alternative simplifies the indicator reaction.

This strategy consists on the formation of gold nanoparticles (AuNPs) by reaction with NADH or NADPH, cofactors of enzymatic reactions catalyzed by dehydrogenases.

In this case, the studied reaction will be the degradation of tropine. In this reaction, the enzyme tropinone reductase produces the oxidation of tropine to tropinone using NAD as a cofactor and giving NADH as a product. At a later stage the generated NADH will react with Au(III) giving rise to purple AuNPs.

Both reactions will be optimized separately and finally coupled into a single system.

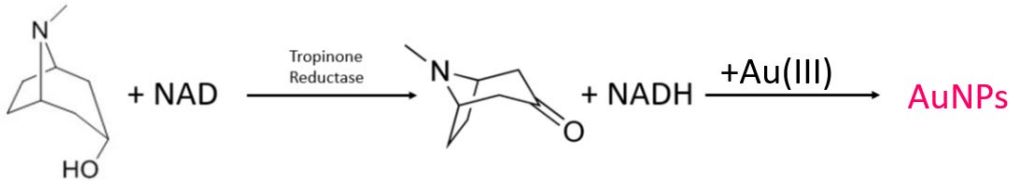

Figure 2. *Tropine oxidation enzymatic pathway, coupled to a subsequent reaction with Au(III).*

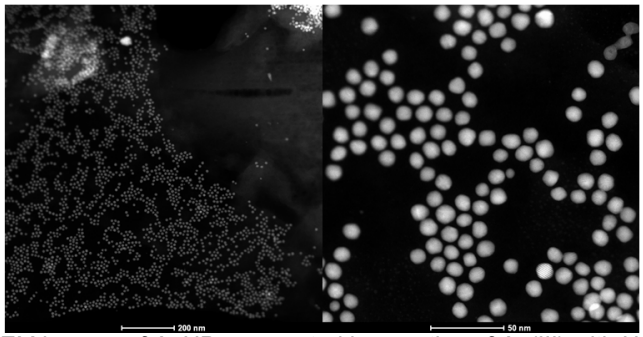

Figure 3. *TEM images of AuNPs generated by reaction of Au(III) with NADH.*

Acknowledgements
This work is part of the I+D+I project PID 2019-105408 GB-I00 supported by MCIN/AEI/10.13039/5011000011033/ and the funding for research groups of the Government of Aragón (E25_20R).

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P20. DETERMINATION OF OPIUM ALKALOIDS IN POPPY SEED INFUSIONS BY EXTRACTION WITH A MAGNETIC MATERIAL BASED ON MESOSTRUCTURED SILICA FUNCTIONALISED WITH β-CYCLODEXTRIN

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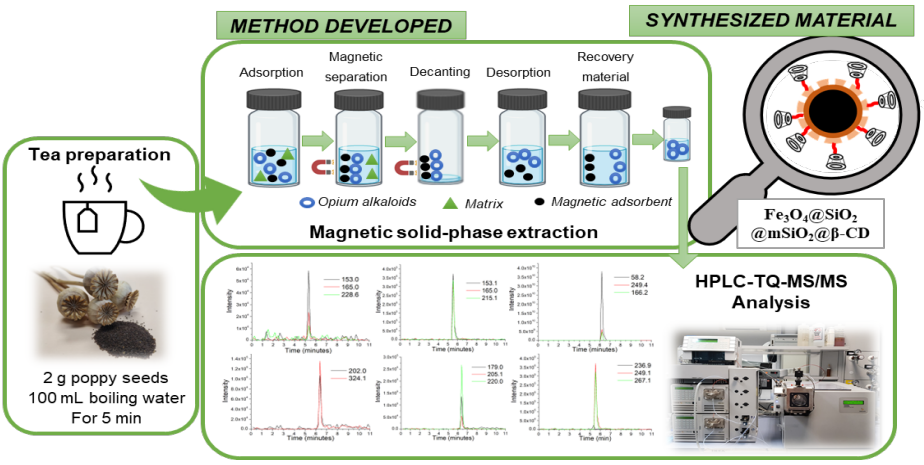
Keywords: *opium alkaloids, poppy seed infusions, magnetic solid-phase extraction, mesostructured silica, β-cyclodextrin, liquid-chromatography tandem mass spectrometry.*

Consumption of poppy seed infusions can cause serious cases of intoxication. To take control measures, it is necessary to develop methodologies to analyze opium alkaloids (OAs) in these infusions[1]. The current trend is to develop sample preparation methods that are faster, and simpler, with the use of new adsorbents and with lower amounts of organic solvents to make them more environmentally friendly. In the present work, a methodology has been optimized for the quantification of six OAs in poppy seed infusions by magnetic solid-phase extraction (MSPE) followed by analysis with liquid chromatography tandem mass spectrometry (HPLC-MS/MS). To do this, first, the synthesis was optimized, and a magnetic compound of mesostructured silica with β-CD (Fe_3O_4@SiO_2@mSiO_2@β-CD) was characterized. Subsequently, the MSPE procedure was optimized, for this purpose, the best conditions in the adsorption step were evaluated by performing adsorption studies in water at different pH (1, 2, 7, 7, 9 and 11) and times (1, 5, 10 and 20 min) in ultrasound (US), obtaining the maximum adsorption at pH 9 in 1 min. Then, optimization of the amount of material required (50 mg) and the elution conditions (2 mL of water/EtOH at 50% with 1% formic acid for 1 min in the US) was carried out. The methodology, once optimized, was successfully validated with low limits of detection and quantification, negligible matrix effect and good recovery values (89-94%). Finally, the developed methodology was applied to the analysis of infusions with four different poppy seeds. The results showed worrying amounts of OAs in one of the infusions considering that the seeds used in that infusion had at least four times the legislated level[2].



Acknowledgements
To the funding of MCIU/AEI/FEDER, UE for the project RTI2018-094558-B-I00.

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P21. ANALYTICAL METHODOLOGY DEVELOPED TO EVALUATE THE DEGRADATION OF OPIUM ALKALOIDS IN POPPY SEEDS WITH GRINDING BASED ON ULTRASOUND-ASSISTED EXTRACTION, PURIFICATION BY SOLID-PHASE EXTRACTION WITH SULFONIC ACID-FUNCTIONALIZED SBA-15 AND HPLC-MS/MS ANALYSIS

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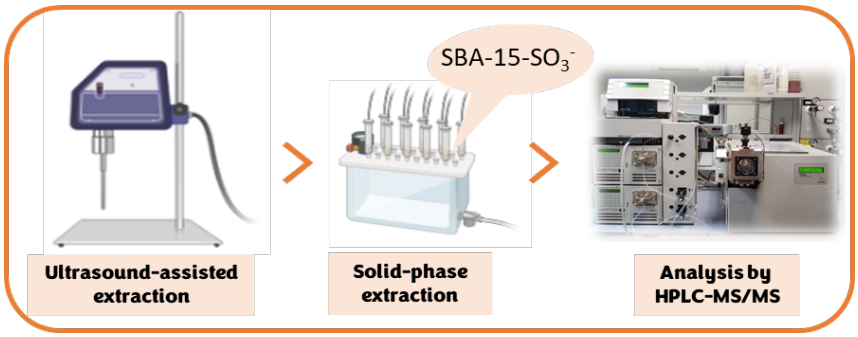
Keywords: *opium alkaloids, ground poppy seed, solid-phase extraction, sulfonic acid-functionalized SBA-15, liquid-chromatography tandem mass spectrometry.*

Nowadays, poppy seeds are increasingly being used to add to various food products because of their good nutritional properties, one of the ways in which they are added is ground. The problem is that the seeds can be contaminated with opium alkaloids (OAs), and consumption in high concentrations can lead to serious cases of intoxication[1]. To date, there is no study that has developed a methodology to analyse ground seeds, and due to the possible degradation that OAs can cause by grinding, it is important to determine their concentrations and even to know the degradation degree to establish a real exposure to consumers[2]. Therefore, the aim of the present work was to develop and validate an efficient, rapid, and environmentally friendly methodology for the quantification of the six main OAs in ground poppy seeds by liquid chromatography coupled to a triple quadrupole tandem mass detector (HPLC-TQ-MS/MS). For this purpose, the ultrasound-assisted extraction (UAE) step was optimised using design of experiments and the complete extraction of OAs was obtained using a lower sample-to-solvent ratio and shorter times than those used with classical magnetic stirring. A purification step by solid phase extraction (SPE) was then optimised to eliminate potential matrix effects that can cause false results and further deterioration of the equipment. For this purpose, a previously synthesised material of silica SBA-15 functionalised with sulphonic groups (SBA-15-SO_3^-) was used, and the conditions of the purification steps (conditioning, loading and elution) were optimised to achieve adequate recovery values with only 25 mg of material. Finally, this methodology was adequately validated in terms of linearity, limits of detection and quantification, precision, accuracy, and selectivity, and successfully applied to the analysis of OAs in ground poppy seeds and to evaluate the degree of degradation of OAs after different grinding conditions.



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P22. HYDROLATES OF BOTANICAL CROPS AS INGREDIENTS IN ECO-COSMETICS

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Keywords: *bioactive compounds, cosmetics, natural extracts, fragrance substances.*

Plants are a source of botanical ingredients with application in cosmetic formulations based on their traditional use. In recent years, growing scientific evidence confirms the cosmetic properties of various extracts and ingredients obtained from plants[1]. The market demands for products that include these phytochemicals-based formulations are increasing. In addition, their use involves the revaluation of co-products and by-products generated in the commercial exploitation of various plants in the agriculture, food and forestry sectors, and searches for complementary ways to use previously cultivated plants as spices or infusions, or even other wild ones. This is an innovative concept that gives extra value and facilitates the approach to a circular economy.

This study covered the following typical Galician organically cultivated botanicals: grelo flowers (*Brassica rapa var. Rapa*), yarrow petals (*Achillea millefolium*), laurel leaves (*Laurus nobilis*), *xesta pudia* flowers (*Genista florida*) and filipendula flowers (*Filipendula vulgaris*). It is important to note that some phytochemical profiles were not analytically characterized in depth, and that specific species were not considered or are totally unexplored. Extracts from these plants contain polyphenols, but also other valuable bioactive compounds such as terpenoids. This work has focused on the analysis of volatile compounds from hydrolates obtained at company level. To extract these substances, we propose a simple and rapid procedure based on solid-phase microextraction (SPME), in which different experimental conditions were tested, followed by subsequent desorption into a gas chromatograph coupled to mass spectrometer (GC-MS). The aim was to determine the aromatic profile of these samples.

In addition, among the compounds identified, common or specific compounds between extracts were searched. The most remarkable properties of the phytochemicals present in the botanicals under study were investigated as valuable and multifunctional ingredients for cosmetic use[2], in order to design new original and ecological cosmetic preparations that respond to the current demands of society. Finally, it was checked if any of the identified fragrance compounds were included in the list of 82 substances declared as contact allergens by the SCCS[3].

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This work was supported by the projects ED431B 2020/06 (Galician Competitive Research Groups, Xunta de Galicia, Spain) and FEADER 2021/058A (Consellería do Medio Rural, Xunta de Galicia, Spain). This study is based upon work from the Sample Preparation Study Group and Network, supported by the Division of Analytical Chemistry of the European Chemical Society. All these programs are co-funded by FEDER (EU).

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P23. APPLICATION OF µSPEED® FOLLOWED BY UHPLC-MS/MS ANALYSIS TO THE DETERMINATION OF PYRROLIZIDINE AND TROPANE ALKALOIDS IN HONEY SAMPLES

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Keywords: *pyrrolizidine alkaloids, tropane alkaloids, microextraction, UHPLC-MS/MS, food safety, honey, µSPEed.*

Pyrrolizidine alkaloids (PAs) and tropane alkaloids (TAs) are natural plant toxins that have recently gained special interest in food safety due to their concerning occurrence in many foods. As a result, many food alerts have notified the presence of these alkaloids at concentration levels higher than the maximum levels established by the competent authorities in food, which could lead to the appearance of chronic diseases or acute intoxications. In general, these alkaloids are produced by plants that grow in fields as weeds and contaminate food crops, leading to their appearance in the production of plant-derived food products. Nonetheless, their occurrence has also been detected in animal-derived food products, such as honey because of pollen dislodge into nectar by the bees during the pollination process[1]. Therefore, due to their potential risk for human health, it is of utmost importance to monitor the occurrence of these alkaloids in food. Accordingly, since PAs and TAs have similar contamination pathways, they can appear as contaminants in the same products, so it seems suitable to develop analytical strategies that enable the simultaneous determination of these two types of toxins. Hence, the aim of this work is to propose a multicomponent microextraction and analysis for the simultaneous determination of 21 PAs and 2 TAs in honey samples. For this purpose, the honey samples were first diluted with 10 mL of H_2SO_4 0.05 M to achieve protein precipitation, and then the sample extract was purified with a novel microextraction technique called µ-SPEed®, which it is an adsorption procedure that uses small sorbent particles (<3 µm) tightly packed in a disposable needle equipped with a pressure-driven valve to withdraw sample flow in a single direction at high pressure (up to 1600 psi)[2]. Different extraction parameters (e.g., type of sorbent, extraction cycles, elution volume) were evaluated and the final optimized procedure involved: the conditioning and activation of a PS-DVB cartridge (4 mg) using 2 aspiration-dispense cycles of water, followed by 2 cycles of H_2SO_4 0.05 M. Then, the sample loading, and finally the elution with methanol. Accordingly, a sustainable and sensitive extraction procedure that meets the “green chemistry principles” was developed, as extraction time of each sample took approximately 5 min and minimal amounts of sample and organic solvent were required per analysis, showing good analytical performance (recovery values >70%). The sample extracts obtained were analyzed by UHPLC-IT-MS/MS in ESI positive mode. The chromatographic separation was performed on a Luna Omega Polar C18 column (set at 30 ºC), and a gradient elution was carried out combining water containing 0.2% formic acid with methanol 0.2% ammonia. The chromatographic separation of the 23 analytes was achieved in less than 15 min. The method was properly validated, and its feasibility was proved by the analysis of different honey samples.

Acknowledgements
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P24. EMERGING OPPORTUNITIES OF MESOSTRUCTURED SILICA-BASED MATERIALS AS SORBENT WITHIN ALKALOIDS DETERMINATION IN FOOD SAMPLES

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Keywords: *mesostructured silicas, post-synthesis, one-pot synthesis, solid-phase extraction, alkaloids, food samples.*

Alkaloids are natural compounds produced by plants as secondary metabolites, and most of them are toxic even at low doses. Today, these alkaloids can enter the food chain as constituents of a wide variety of foods obtained from plant sources, such as seeds, cereals, honey, teas among others. The presence of these compounds can pose a potential risk to human health. Therefore, the control of these residues in food products is of extreme importance to ensure food safety, which has led to the need to develop rapid, sensitive, and selective analytical methodologies for the determination of alkaloids in food samples. Due to the complexity of such matrices, an additional clean-up step is often necessary to eliminate interferences as for improving the sensitivity and precision of the method. In this sense, the development of new materials for their application as sorbents in sample preparation is one of the great challenges in analytical chemistry, as these materials can play an important role in pre-concentration and selective extraction tasks. Thus, ordered mesostructured silicas (OMS) present themselves as a promising alternative to classical sorbents. This is due to their desirable characteristics, like a highly ordered and size-controlled structure, a high surface area, and a large pore volume. In addition, they have a high flexibility to functionalisation that allows the introduction of different functional groups on the surface, which could enable the efficient and selective extraction of target analytes from food matrices. This work aims to show the achievements in the preparation of OMSs and other hybrid silicas, and their application in sample preparation for the determination of alkaloids in food samples.

In this sense, different OMS (SBA-15 and HMS) functionalised by post-grafting (pg) with sulphonic acid groups (SO_3^-) have been synthesised to obtain materials with strong cation exchange retention mechanism, named SBA-15-SO_3-pg and HMS-SO_3-pg respectively. With the same objective, two new materials functionalised with sulphonic groups were synthesised, but this time prepared by one-pot (op) synthesis (SBA-15-SO_3-op and HMS-SO_3-op). On the other hand, with the intention of fabricating a material with mixed-mode behaviour, an SBA-15 with sulphonic and cyanopropyl groups (SBA-15-SO_3-CN) has been biofunctionalised. Finally, a mesostructured imprinted silica (MIS) has also been synthesised using SBA-15 as a support, (3-isocyanatopropyl)triethoxysilane (ICPTES) as functional monomer, and morphine as a template (SBA-15-MIS-MF) in order to obtain a selective material. The resulting materials were extensively characterised by powder X-ray diffraction, TEM, SEM, N_2 adsorption, ^{29}Si-NMR and ^{13}C-NMR spectrometry, elemental analysis, etc., to obtain the necessary information on structural, compositional, and porosity parameters. The functionalised materials were evaluated as SPE sorbents for the extraction and preconcentration of tropane (TAs) and/or opium (OAs) alkaloids. So, the post-grafting materials (SBA-15-SO_3-pg and HMS-SO3-pg) have been successfully applied to determine TAs in flours[1], aromatic herbs[2] and spices[3]. On the other hand, HMS-SO3-op have been employed favourably as SPE sorbent for the chiral determination of (±)-hyoscyamine (TAs) in herbal infant infusion samples. The biofunctionalized material (SBA-15-SO_3-CN) has been applied for the simultaneous determination of TAs and OAs. Finally, the SBA-15-MIS-MF material will be evaluated as a selective sorbent for the extraction of OAs. The results obtained so far suggest that the prepared materials are a promising sorbent for SPE, and can be a good alternative to commercial sorbents for the determination of TAs in food.

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P25. DEVELOPMENT OF A MOLECULARLY IMPRINTED POLYMER FOR SELECTIVE EXTRACTION OF TROPANE ALKALOIDS IN HONEY SAMPLES PRIOR TO THEIR ANALYSIS BY LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY

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Keywords: *tropane alkaloids, molecularly imprinted solid-phase extraction, HPLC-QqQ-MS/MS, food samples, honey.*

Tropane alkaloids (TAs) are a group of more than 200 compounds synthesized as secondary metabolites by a wide variety of family plants such as Solanaceae, Brassicaceae, Erythroxylaceae, Euphorbiaceae or Convolvulaceae. Atropine and scopolamine are the most studied TAs and these toxics could be found in certain foods as a result of cross-contamination with TA-producing plants since they grow in fields like weeds. Honey is a natural foodstuff produced by bees that could be contaminated by these insects as a consequence of the pollination of flowers that contains TAs. The extraction is an important step in the analysis of these compounds, especially in complex food samples and one of the most commonly used techniques is the solid-phase extraction (SPE)[1]. Actually, to increase the selectivity of the extraction, new materials such as molecularly imprinted polymers (MIPs) have been developed and evaluated for TA analysis in food samples[2].

In this work, two MIPs were synthesized by precipitation polymerization using scopolamine as template, ethylene glycol dimethacrylate (EGDMA) as a cross-linker, 2,2-azobisisobutylnitrile (AIBN) as initiator, acetonitrile as reaction solvent and 4-vinylpiridine (4-VP) or methacrylic acid (MAA) as functional monomers. To remove the template of the polymers, it was carried out a Soxhlet extraction using methanol-acetic acid (80/20 v/v) as solvent. In the same way, two polymers with 4-VP and MAA were synthesized following the same procedure but without the addition of the template (non-imprinted polymers or NIPs). Both materials were characterized with different structural techniques such as scanning electron microscope (SEM) to determine the morphology of the molecules or BET analysis to measure the surface area and the porosity of the MIPs.

To evaluate the extraction capacity of these polymers, different studies in SPE (MISPE) were carried out. For this, SPE cartridges have been packaged with different amounts of polymer (MIP or NIP) and the extraction process was carried out under different loading and elution conditions using scopolamine and atropine at different concentrations. After the MISPE process the extracts were analyzed with a high-performance liquid chromatography coupled to triple quadrupole mass spectrometry detector (HPLC-QqQ-MS/MS) with an electrospray ionization (ESI) operating in positive mode and using a C18 column (set at 30 ºC). High recovery percentages were observed for both analytes (70-120%) and the best results were obtained with the following conditions: 25 mg of polymer (4-VP-MIP and MAA-MIP), acetonitrile as loading solvent and methanol/acidified water (1% formic acid) in a 60/40 (v/v) proportion as elution solvent. The methodology will be applied to determine TAs in honey in order to evaluate the interference effect and select the more efficient MIP. Finally, the method will be properly validated and applied to the analysis of different commercial honey samples since actually there are few works that analyze TAs in animal-derived foods and with the aim of expanding knowledge about the intake of these alkaloids.

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P26. OPTIMIZATION OF A GREEN EXTRACTION PROCEDURE TO OBTAIN POLYPHENOLIC COMPOUNDS FROM THE WINE INDUSTRY BY-PRODUCTS

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Keywords: *GRAS solvents, LC-MS/MS, MSAT, antioxidant activity, ethyl lactate, grape marc, natural extracts, polyphenols.*

EU agri-food export trade has been led in the last decade by the wine sector, being one of the most important agro-industrial activities in the world[1]. However, more than 5 million tonnes of marc, the main by-product of this industry, are generated each year. Several studies have addressed the reuse of this derivative based on its bioactive power represented by its high polyphenolic content, generating small doses of extract with potential incorporation in the food industry, cosmetics, and its reincorporation in the wine sector[2]. The main objective of this work is focused on the evaluation and optimization of a scalable process with minimum energy requirements MSAT (Medium Scale Ambient Temperature) for obtaining polyphenolic extracts from the white grape marc employing generally recognized as safe (GRAS) solvents including propylene glycol (Pg), ethanol (EtOH) and ethyl lactate (EtLc), as well as their hydro-organic mixtures. In a first approach, through a response surface matrix, the operational parameters, extractive volume, marc mass and its ratio with a dispersant were optimized, looking for an efficient process able to generate higher volumes of extract and bioactivity. In this way, the highest total polyphenolic content (5918 mgGAE·L^{-1}) and antioxidant activity (44 mMTE) values were achieved at a maximum operational volume of 100mL. On the other hand, to obtain an extract suitable for nutraceutical purposes, the affinity profile towards the main polyphenolic compounds and carbohydrates was explored by HPLC-MS/MS and UHPLC-QTOF, respectively. The overall response of the bioactive activity as well as the individual phenolic profile was obtained by using EtLc>EtOH>Pg, whereas the isovolumetric mixture EtLc/Water showed the highest concentrations for the polyphenols: quercetin (5.4 mg·L^{-1}), quercetin-3-glucuronide (22.4 mg·L^{-1}), kaempferol (1.0 mg·L^{-1}) and quercetin-3-glucoside (26.0 mg·L^{-1}) together with a lower concentration of reducing sugars, favouring their potential use of the extracts in a solid formulation.

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P27. MATRIX SOLID-PHASE DISPERSION AND ULTRASOUND-ASSISTED EXTRACTION IN THE EXTRACTION AND POLYPHENOLIC CHARACTERISATION FROM FIVE VARIETIES OF BROWN ALGAE

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Keywords: *Adriatic Sea macroalgae, polyphenols, matrix solid-phase dispersion, ultrasound-assisted extraction, green chemistry*.

Among the broad group of phytochemical compounds, polyphenolic structures are of special interest due to their bioactive capacity, being important antioxidants associated with beneficial effects on health[1]. Although the potential of brown algae to contain these compounds is well known, research on their polyphenolic potential is still scarce[2]. In this work, the total polyphenolic content of five brown algae: *Dictyota dichotoma, Cystoseira barbata, Cystoseira spicata, Ellisolandia elongata* and *Sargassum sp.* is evaluated and characterised, as well as the effect of the extractive solvent on their recovery. In this way, the solvents methanol, ethanol, and water, were selected as extractants in the application of the techniques Matrix Solid-Phase Dispersion (MSPD) and ultrasound-assisted extraction (UAE) (Fig.1), using as indicators of the extractive efficiency the bioactive properties total polyphenolic content (TPC) and antioxidant activity (AA), and the individual polyphenolic profile analysed by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). The results showed a wide range of TPC from 0.20 mgGAE·g^{-1} for the methanolic extract of *E. enlongata* to 38 mgGAE·g^{-1} corresponding to the aqueous ethanolic extract of *Sargassum sp.* In general, the isovolumetric ethanolic ratio shows a better overall recovery of phenolic compounds, the macroalga *D. dichotoma* being the exception, improving its TPC by 20% when methanol is applied as extractant. At the same time, a higher extractive and bioactive efficiency of the extracts is obtained using UAE compared to MSPD. According to their individual profiles, hydroxybenzoic acid and some of its derivatives, as well as other bioactive compounds of interest, have been detected in all the species studied, providing a better knowledge of the bioactive content of these macroalgae species, and their potential application to the nutraceutical sector.

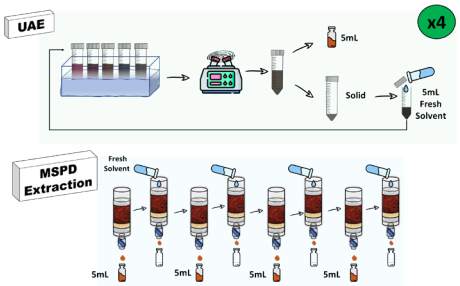

Figure1. *Comparative analysis of matrix solid-phase dispersion and ultrasound-assisted extraction techniques in the sequential extraction of 5 varieties of brown algae*.

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P28. SOLID-LIQUID EXTRACTION AND DISPERSIVE LIQUID-LIQUID MICROEXTRACTION AND GAS CHROMATOGRAPHY WITH FLAME IONIZATION DETECTION FOR THE DETERMINATION OF INSECT CUTICULAR HYDROCARBONS

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Keywords: *cuticular hydrocarbons, dispersive liquid-liquid microextraction, gas chromatography.*

The cuticular cover of insects plays a key role both in their protection from the environment and in chemical communication between and within species. This is made possible by the characteristic cuticular composition of the species, which is mainly composed of lipids such as hydrocarbons and wax esters and, to a lesser extent, fatty acid esters, triacylglycerols, alcohols, aldehydes, ketones and free fatty acids. Forensic science exploits this attribute to estimate post-mortem intervals, as the cuticular hydrocarbon profile is closely related to the stage and species of the insect[1,2].

The aim of this work was the development of a high-sensitive analytical methodology for the qualititative and quantitative determination of the cuticular hydrocarbons composition of insects, providing a reliable tool for forensic investigation in the discrimination between morphologically similar specimens. For this purpose, a sample treatment combining solid-liquid extraction (SLE) and dispersive liquid-liquid microextraction (DLLME) procedures has been optimized using specimens of Diptera order, specifically from the Calliphoridae family (fixed in 70:30 ethanol:water solution). Under the finally selected conditions, isolation of the cuticular hydrocarbons was carried out by adding 2 mL of acetonitrile to one specimen and the mixture was vortex stirred for 5 min. The organic extract was used as dispersant solvent in the subsequent DLLME stage, being combined with 75 µL of chloroform and injected into 10 mL of water. The dispersion of chloroform phase in multiple fine microdrops allowed the rapid extraction of the cuticular hydrocarbons, being the enriched phase collected after centrifugation for 5 min at 3000 rpm. The SLE-DLLME organic extracts were analyzed using gas chromatography with flame ionization detection, providing detection of the different cuticular hydrocarbons at very low concentration levels.

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P29. A COMPARATIVE STUDY ON THE ANALYTICAL PERFORMANCE OF µSPEED AND µQuEChERS FOLLOWED BY UHPLC-PDA FOR THE SIMULTANEOUS DETERMINATION OF PESTICIDES IN WASTEWATER

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Keywords: *pesticides, µSPEed, µQuEChERS, multi-residue separation, ultra high-performance liquid chromatography, wastewater samples.*

Residues of pesticides can be present in food (fruits, vegetables) and environmental samples (water and soil) due to their wide use in protecting crops from different aggressions and obtaining good yields in less time. Therefore, determining the presence of pesticides in these samples is of utmost importance to ensure that their levels are below the established maximum residue limits (MRLs). Different extraction techniques have been used to develop sensitive analytical methodologies for pesticide determination in samples of interest. In this context, microextraction techniques have emerged as green analytical approaches since they reduce the environmental impact associated with conventional extraction techniques due to the low volumes of reagents and samples required.

In this work, a µSPEed procedure, operated by a semiautomatic electronic syringe, digiVol®, was optimized and validated for the simultaneous extraction of eight pesticides (paraquat, thiabendazole, asulam, picloram, ametryn, atrazine, linuron, and cymoxanil) from residual waters. Eight different sorbents were tested, and the best results were obtained with C_{18} sorbent. This microextraction procedure was carried out with the minimum volumes of solvents and samples (250 µL methanol activation, 250 µL water equilibration, 2×250 µL sample loading, and 2×50 µL methanol elution), with an extraction time of less than 5 min. The analytical performance of this microextraction technique was compared with a µQuEChERS methodology previously optimized using a simple and quick procedure that does not require any sophisticated laboratory equipment. Both microextraction procedures were followed by a 7.5 min UHPLC chromatographic separation equipped with a PDA detection system and using a 1.8 µm HSS column and a mobile phase consisting in a gradient of acidified acetonitrile. Satisfactory analytical performance was obtained for both proposed methodologies, which were applied to the determination of the target analytes in residual waters.

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P30. NATURAL DEEP EUTECTIC SOLVENTS AS A GREEN TOOL FOR THE EXTRACTION OF FLAVANONES FROM ORANGE BY-PRODUCTS

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Keywords: *orange waste, flavanones, antioxidant capacity, natural deep eutectic solvents, ultrasound-assisted extraction.*

The agro-food industry, specifically fruit and vegetable processing, generates a significant amount of waste, with the citrus industry discarding around 50-60% of the total fruit weight. Orange byproducts have the potential to be a valuable source of high-added-value products and energy, as they contain a substantial quantity of biologically active secondary metabolites, such as phenolic compounds. To address the environmental concern associated with traditional organic solvents, natural deep eutectic solvents (NaDES) have emerged as a cost-effective and environmentally friendly alternative. In this study, a green extraction approach combining ultrasound-assisted extraction (UAE) and NaDES was developed for extracting antioxidant flavanones from orange byproducts. Five different NaDES were evaluated to determine the most effective solvent for flavanone extraction. The extracts were analyzed for their antioxidant capacity and total proanthocyanidin contents. Experimental findings revealed that choline chloride-lactic acid (1:3) NaDES exhibited the highest efficiency in recovering antioxidant proanthocyanidins and flavanones from the extracts. To optimize the UAE process using NaDES, a Box-Behnken experimental design was employed, considering water percentage (0-30%, v/v), ultrasound amplitude (30-60%), and extraction time (1-20 min) as the main parameters. The optimal extraction conditions were determined to be 3% (v/v) water, 60% ultrasound amplitude, and 14 min of extraction time. The flavanones present in the extracts were identified and quantified using high-performance liquid chromatography with a diode array detector and quadrupole-time-of-flight mass spectrometry. The results demonstrated that both ultrasound amplitude and extraction time significantly influenced the extraction efficiency of antioxidant flavanones from orange waste.

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P31. COMBINATION OF GREEN TECHNOLOGIES FOR THE EXTRACTION OF ANTIOXIDANT PHENOLIC COMPOUNDS FROM ORANGE BY-PRODUCTS

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Keywords: *antioxidant capacity, enzyme-assisted extraction, orange byproducts, phenolic compounds, ultrasound-assisted extraction.*

The citrus processing industry focuses mainly on juices production, generating large amounts of waste which elimination is associated to high economic costs. The revalorization of by-products has become a good strategy to reduce food-processing losses and set a more sustainable production and consumption system. Citrus by-products have been considered an economic source of biological and commercial compounds that foster zero-waste models[1]. One of the main challenges in using by-products is the recovery of bioactive compounds as it often requires energy-intensive processes and the use of organic solvents. These processes can be toxic and non-environmentally friendly[2]. Therefore, this study presents a novel approach for extracting antioxidant phenolic compounds from orange (*Citrus sinensis*) pomace. The strategy involves the combination of sustainable extraction techniques such as ultrasound-assisted extraction (UAE) and enzyme-assisted extraction (EAE). UAE-EAE was compared with individual UAE and EAE to evaluate the efficiency of phenolic compound extraction from this matrix. In addition, different enzymes were tested, Depol and Promod being identified as the most promising ones for the extraction process. Box-Behnken experimental designs were employed to determine the optimal UAE-EAE conditions for enzyme concentration (50-100 µL/g), ultrasound amplitude (30-60%), buffer pH (6.00-10.00), and extraction time (1-15 min). The total phenolic (measured by the Folin-Ciocalteau method) and proanthocyanidin (determined by DMAC and HCl/butanol assays,) contents and antioxidant capacity (evaluated through ABTS, DPPH, and hydroxyl radicals scavenging assays) were measured. The optimal conditions for UAE-EAE using Promod enzyme were an enzyme concentration of 100 µL/g, 30% amplitude, pH 8.4, and 1 min extraction time; while when using Depol enzyme, an enzyme concentration of 100 µL/g, 30% amplitude, pH 8.0, and 8 min extraction time. In addition, phenolic compounds present in the optimal extracts were determined using HPLC-DAD. Flavanones were the main phenolic group present in the extracts of orange pomace. Optimal extraction conditions employing UAE-EAE to recover phenolics from orange pomace were compared with conventional extraction. The results indicated that EAE-UAE was a suitable extraction method to obtain phenolic compounds from orange pomace having extracts with a higher content of proanthocyanins and antioxidant capacity than those obtained by conventional extraction.

Acknowledgments
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P32. ULTRASOUND-ASSISTED EXTRACTION AND CYCLODEXTRIN ENCAPSULATION OF PHYTOSTEROLS FROM CHERIMOYA SEEDS

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Keywords: *cherimoya seeds, cyclodextrins, encapsulation, GC-MS, phytosterols, ultrasound-assisted extraction.*

The current global overpopulation led to the necessity of producing large quantities of food, resulting in the generation of significant by-products and waste throughout the agricultural production, food processing, distribution, and consumption processes. These food wastes and by-products often contain valuable nutrients and bioactive compounds, such as dietary fiber, phytosterols, polyphenols, and phenolic acids[1]. For instance, phytosterols are natural sterols and stanols present in the non-saponifiable fraction of vegetable oils[2]. Studies have shown that they decrease serum cholesterol levels in humans reducing the risk of coronary heart disease, cerebrovascular disease, and peripheral vascular disease, as well as cancer and diabetes[2,3]. Therefore, this work proposes to enhance the value of food waste by recovering bioactive lipidic compounds, such as phytosterols, from cherimoya seeds. With this aim, a method was developed and optimized to extract phytosterols by a sustainable extraction technique, such as ultrasound-assisted extraction (UAE) using biobased solvents. Since solvent selection is one of the critical factors in phytosterol extraction-encapsulation, preliminary experiments were conducted to determine the best extraction-encapsulation solvents. Finally, ethanol and ethyl acetate were chosen to successfully extract-encapsulate the phytosterols. The phytosterols present in the extracts were determined by gas chromatography coupled with mass spectrometry (GC-MS). A Box-Behnken experimental design was employed to optimize the main parameters in UAE, including the solid-to-solvent ratio (40-250 mg/mL), extraction time (5-20 min), and ultrasound amplitude (30-60%). The optimal extraction conditions were 33% of amplitude, 61 mg/mL of solid-to-solvent ratio, and 18.5 min using ethyl acetate; and 60% of amplitude, 146 mg/mL of solid-to-solvent ratio, and 13.00 min using ethanol. Ethyl acetate, under the optimal extraction conditions, was able to extract a greater amount of phytosterols from cherimoya seeds, with β-sitosterol being the predominant compound, followed by campesterol and stigmasterol. An encapsulation strategy based on the use of β-cyclodextrin and ultrasound was developed, which protects the bioactive compounds and improves their water solubility and their sustained compound-releasing behavior. Phytosterol-cyclodextrin powder was characterized by Attenuated Total Reflection Fourier Transform Infrared spectroscopy (ATR-FTIR) and the encapsulated phytosterols were determined by GC-MS analysis. Results indicated that the extraction-encapsulation of phytosterols from cherimoya seeds in cyclodextrins could provide a sustainable alternative for important applications in the food and pharmaceutical industries.

Acknowledgments
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P33. GRAPHENE QUANTUM DOTS: A NOVEL APPROACH TO IMPROVE THE PHOTOSTABILITY AND ANTIOXIDANT CAPACITY OF *TRANS*-RESVERATROL IN FOOD SYSTEMS

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Keywords: *light stability, stereoisomeric inhibition, carbon-based nanomaterial, additives, food.*

Resveratrol (RESV) is a well-known non-flavonoid polyphenol, a member of the stilbenes family, that has attracted considerable interest due to its health-promoting properties, including cardioprotective, neuroprotective, antioxidant and anti-inflammatory activities[1]. RESV can be found in the skin of grapes, blueberries, raspberries, and blackberries but the mentioned health benefits are mainly associated with the *trans*-isomer. However, the efficiency of *trans*-RESV is limited due to its low chemical stability[2]. *Trans*-RESV is quickly transformed into *cis*-RESV, which is a less biologically active isomer, when exposed to different sources of light radiation. To maximize the bioactive properties of RESV, it is advisable to protect *trans*-isoform from light to prevent or delay the quick isomerization as far as possible.

A new application provided by a carbon-based nanomaterial, specifically graphene quantum dots (GQDs), involves the adsorption of RESV on their surface in such a way that it confers photostability to the polyphenol, producing a considerable inhibition in the conversion of *trans*- to *cis*-RESV when irradiated with UV light[3] (**Figure 1**). To monitor this effect, an analytical method based on the capillary electrophoresis technique was used to determine the proportion of isomers at any given time. To address concerns regarding the use of GQDs in food products, toxicity assays were conducted and revealed no adverse effects on the normal growth of model microorganisms tested by the presence of the nanomaterial. Furthermore, the adsorption of RESV on the carbonaceous materialincreases the antioxidant capacity of the bioactive, which adds to its healthy properties. The efficacy ofthis process was evaluated in several RESV-rich beverages and dietary supplements that weresubjected to controlled radiation times, resulting in a significant slowing down of isomerization, even more than eleven times. This confirms the potential of GQDs to be an effective *trans*-RESV vehicle to complement food systems.

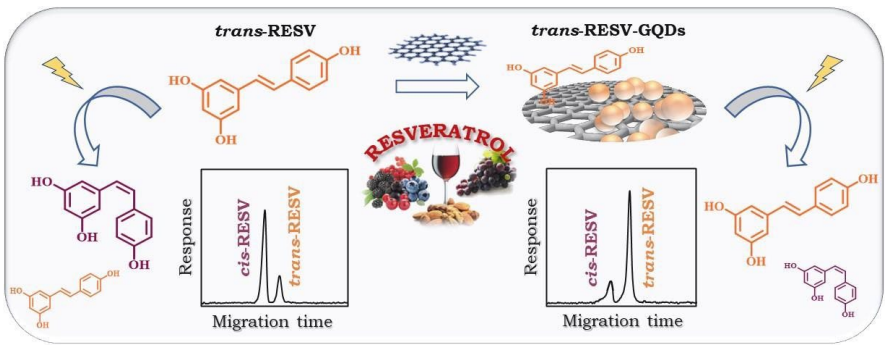

Figure 1. *Pictorial representation of trans-RESV adsorption on GQDs for largely inhibition its isomerization.*

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P34. DETERMINATION OF ACARICIDES IN BEE PRODUCTS BY USING GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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Keywords: *acaricides, bee pollen, beeswax, GC-MS, honey, sample treatment, method validation.*

In recent years a series of food alerts have been issued related to the detection of contaminants such as pollutants, antibiotics, or pesticides in bee products around the world. Pesticides (herbicides, acaricides, and insecticides) that are used both in agriculture and livestock can reach the beehives and subsequently their related products like honey, beeswax, or bee pollen through the pollination process. Honeybees travel many kilometres to collect pollen and nectar, so contamination cannot be avoided only by controlling the areas near the hives. In addition, other pesticides, especially those employed to fight against *Varroa destructor* like the acaricides bromopropylate, coumaphos, and τ-fluvalinate, can produce direct contamination to the beehives because they are applied directly to the honeycombs. Different options for the use of acaricides, highly conditioned by the form of application, the climatic conditions, as well as the health status of the beehive, also compromise their effectiveness. This leads to the frequent application of doses higher than those recommended, and there is a high probability that acaricide residues appear in the different beehive products. Indeed, acaricide residues have been found in honeys from different countries, and maximum residue levels have been established by regulatory agencies to protect the consumer´s health. Therefore, the development of specific and sensitive methodologies for the determination of acaricides in bee products is justified.

The main goal of this research was to propose alternative methods for determining simultaneously seven of the most frequently detected acaricides (atrazine, chlorpyrifos, chlorfenvinphos, α-endosulfan, bromopropylate, coumaphos, and τ-fluvalinate) in beeswax, bee pollen and honeys from different botanical origins (multifloral, rosemary and heather) by using gas chromatography-mass spectrometry (GC-MS). We developed a GC-MS method that was applied with slight modifications to all bee-related matrices. Moreover, we wanted to focus our attention in proposing efficient, simple, economic, and fast sample treatments. We addressed this issue providing the best performance in terms of extraction efficiency (recoveries) and significance of the matrix effect for determining the selected acaricides in the different bee products. In addition, some of these objectives (reduction of time and cost, number of steps, and amount of reagents) as well as searching for environmentally friendly reagents/solvents are in good agreement with the principles of the green analytical chemistry. Further goals of the present study were to validate the proposed methods for each bee product, and to analyze samples from different Spanish regions.

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P35. DETECTION OF BIOLOGICAL MARKERS OF OXIDATIVE STRESS IN OWL PLASMA AND BLOOD CELLS SAMPLES BY CAPILLARY ELECTROPHORESIS

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Keywords: *biological markers of oxidative stress, capillary electrophoresis, solid phase extraction, owl plasma and blood cells samples.*

Oxidative stress is defined as the imbalance between reactive oxygen species (ROS) and the ability of the body to counteract with antioxidants[1]. Though, the formation of ROS is the main cause of the cellular damage occurrence in the organism. Oxidative stress mechanism can be caused by multiple factors, exogenous (high temperatures, ultraviolet radiation, food and chemical) or endogenous (mitochondrial action), which action, regardless of the type of source, might be harmful to living being[2]. In order to understand the mechanism of action of ROS it is required analytical methodologies and specific biological samples to carry out their detection and analysis. Distinct methods have been used to measure the extent and nature of oxidative stress, ranging from oxidation of DNA to proteins, lipids, and free amino acids.

The present work describes a new method of solid phase extraction (SPE) coupled to capillary electrophoresis with a diode array detector (CE-DAD), for the analysis of five biological markers of oxidative stress: 4-Hydroxynonenal (4-HNE), 8-Hydroxy-2'-deoxyguanosine (8-OHdG), 3-Chloro-L-Tyrosine (CT), 3-Nitro-L-Tyrosine (NT) and L-Tyrosine (Tyr). The running buffer used during this work was Ammonium Acetate (AmAc) (30 mM, pH = 9.3) with 25% of methanol. The limits of detection and quantification of the method ranging from 0.16 to 0.28 µg mL^{-1} and from 0.56 to 0.94 µg mL^{-1}, successively. Furthermore, precision values varied from 0.73% to 0.84% and from 3.22% to 4.68% were calculated for the migration times and for the responses of the studied analytes, respectively. In addition, these compounds were detected in owl plasma and blood cells samples using a solid phase extraction (SPE) method with a good extraction recovery interval varying between 85 and 98%.

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P36. STUDY OF THE VARIATION OF VOLATILE COMPOUNDS IN PEACH DURING RIPENING USING HEADSPACE GAS CHROMATOGRAPHY COUPLED TO MASS SPECTROMETRY

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Keywords: *gas chromatography-mass spectrometry, peach, headspace, volatile compounds, ripening.*

The peach is an edible fruit of the genus Prunus. Because of its juicy flesh, rich flavour and high nutritional value, peach is a very popular fruit on the market, with the largest harvests in China, Spain and Italy. There are many varieties, differing in size, shape, flesh colour, seeds and skin type[1]. In recent years there have been complaints from consumers about the deterioration of the quality of this fruit, which has been related to the early harvesting promoted for economic reasons[2].

Flavour, which influences both the appearance and taste of the fruit, is one of the most important factors in assessing the quality of peaches[3]. Volatile organic compounds (VOCs) present in peaches have been the subject of extensive research and more than 100 VOCs have been identified in this fruit. The most common are aldehydes, alcohols, lactones, terpenes and esters[1]. Genetic origin and the part of the fruit analysed are some of the factors influencing the difference in VOCs concentration, but the most important factor is the degree of ripening. In the early stages, a higher concentration of 6-carbon compounds was found, whereas in the later stages, linalool, lactones and benzaldehyde were more abundant[2]. The study of the different chemical compounds present in peaches at different stages of ripening may provide a method to guarantee the high quality of this fruit.

In this work, an untargeted metabolomics strategy based on the monitoring of VOCs in peaches using headspace-gas chromatography coupled to mass spectrometry (HS-GC-MS) is proposed as a tool to determine the variation in the concentration of VOCs during the ripening process. A fast and simple sample treatment is proposed, consisting of weighing only 2 g of peach puree, containing pulp and skin, together with 0.75 g of NaCl. This mixture is incubated at 120 °C for 15 min before the injection of 1500 µL in 1:10 split mode for GC-MS analysis. Samples were collected during the peach harvest season from agricultural plots located in Jumilla (Region of Murcia, Spain). Peaches of two different varieties were collected from the same tree every 4 days, giving a total of 10 samples for each variety at different stages of ripeness. GC-MS analysis provided a total ion chromatogram with a large number of markers, many of which were identified by MS-DIAL. Some of the identified markers were also quantified to determine how their concentration varied. According to preliminary studies, there is a tendency for the concentration of C6 compounds and aldehydes to decrease and for the concentration of esters and linalool to increase during the later stages of ripening.

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P37. STUDY OF ATROPINE ENANTIOMERS RACEMIZATION IN HERBAL INFUSIONS FOR INFANTS BY CHIRAL ANALYSIS WITH HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY COUPLED TO TAMDEN MASS SPECTROMETRY

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Keywords: *atropine enantiomers, (-)-hyoscyamine, racemization process, chiral separation, HPLC-MS/MS, solid phase extraction, hybrid mesostructured silicas as sorbent.*

The interest in natural toxins produced by plants has increased in recent years due to their toxicity and their impact on food safety, as they can appear as contaminants in varying quantities for various reasons. Among these toxins, atropine belongs to the group of tropane alkaloids, which are produced as secondary metabolites by various plants including *Datura stramonium*, *Atropa belladonna* and *Brugmansia* spp. Atropine is a racemic mixture of (+)- and (-)-hyoscyamine, however, only the (–)-hyoscyamine has high toxicity, such as inhibition of muscarinic acetylcholine receptors in the central and autonomic nervous systems. (–)-Hyoscyamine naturally occurs in plants, but this enantiomer presents some instability and can naturally undergo a racemization process over time, so both enantiomers can occur in variable proportions. Regulation (EU) 2021/1408 sets maximum limits for atropine of 1 µg/kg for cereal-based foods and 0.20 µg/kg for plant infusions (liquid) without differentiating enantiomers, as there are few studies in foods where enantioseparation of atropine has been achieved. However, it is recommended to develop chiral analytical methods to assess the actual intake of each enantiomer and to provide data on its possible racemization to improve current regulation[1,2].

This work presents the development of a method for the chiral analysis of atropine by liquid chromatography in infant herbal infusions. Three chiral stationary phases, amylose tris-(3,5-dimethylphenylcarbamate) (Chiralpak® AD-H), cellulose tris-(3,5-dimethylphenylcarbamate) (Lux Cellulose-1) and amylose tris-(5-chloro-2-methylphenylcarbamate) (Chiralpak® AY-3), have been evaluated using polar (100% methanol, ethanol, 2-propanol, acetonitrile or their mixtures), normal (hexane with methanol, ethanol or 2-propanol as organic modifier) and reverse phase (water with acetonitrile) with basic additives (diethylamine (DEA) and triethylamine (TEA)). The best chiral separation was achieved with Chiralpak® AY-3 column using ethanol/DEA (100/0.05, v/v) as the mobile phase, at a flow rate of 0.6 mL/min and 30 ˚C of temperature showing a good chiral resolution (Rs = 1.6) in an analysis time of 8 min.

For pre-concentration and clean-up of the samples a solid-phase extraction (SPE) procedure was developed. For this, four laboratory-synthesised mesostructured silicas functionalized with sulphonic groups (denoted by SBA-15-SO_3^-(g), SBA-15-SO_3^-(co), HMS-SO_3^-(g) and HMS-SO_3^-(co)) and a commercial one, were evaluated to select the best extraction material to use. The best results were obtained using 50 mg of HMS-SO_3^-(co) showing recoveries close to 100%, a method quantification limit (MQL) of 0.08 µg/L and a good precision in terms of repeatability and reproducibility (RSD < 7%) for both enantiomers. The methodology was applied to the chiral analysis of infant herbal infusions and it was discovered that three of the samples presented contamination with both atropine enantiomers at a concentration of 0.3 µg/L, which exceeds the legal limits, with a ratio of 40% (+)-hyoscyamine and 60% (–)-hyoscyamine, demonstrating that racemization can be produced and measuring both enantiomers as atropine it overestimate the exposure to (–)-hyoscyamine.

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P38. DEVELOPMENT OF A METHOD TO DETECT ADULTERATED OREGANO SAMPLES USING GAS CHROMATOGRAPHY COUPLED TO ION MOBILITY SPECTROMETRY

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Keywords: *oregano adulteration, ion mobility spectrometry, gas chromatography.*

Oregano is often adulterated for economic reasons. This fraud mainly consists of adding other species with lower commercial value such as olive leaves[1,2]. In order to ensure the quality of oregano, an analytical method based on the analysis of the volatile organic compounds (VOC) profile obtained by headspace gas chromatography coupled to ion mobility spectrometry (HS-GC-IMS) was developed and validated. The advantages of IMS such as high sensitivity, fast response, and low cost of operation, combined with the lack of sample treatment, make the proposed methology a very useful and robust tool for its transfer to the agri-food industry.

For method optimization polar and non-polar were columns were invetigated selecting the last one for its higher resolving power. In addition, sample amount, incubation time and temperature, and drift tube temperature for the IMS were optimized. The optimizated method consisted of incubating 0.25 g of sample at 70 °C for 10 min and a 750 µL aliquot of the headspace was injected by a heated syringe into the heated injector (70 °C) in splitless mode. The oven program was set as follows: initial temperature of 50 °C held 4 min, which was increased from 50 °C to 130 °C at 10 °C min^{-1} and held 130 °C for 8 min (total run 20 min). Then, analytes were driven to the IMS module and ionized by a Tritium source at atmospheric pressure in a positive ion mode. Drift tube operated at 90 °C using nitrogen as drift gas at a constant flow of 150 mL min^{-1}.

The analytical method was used to analyze pure oregano, and samples adulterated with two types of olive leaves. A total of 31 VOCs were identified in the analyzed samples, although only 14 compounds could be quantified. In order to find the best calibration curves, logarithmic and Boltzmann´s equations built from the signals of the protonated monomer, proton-bound dimer, or the sum of both signals, were evaluated. Finally, the precision of the method was evaluated in term of repetability and intermediate precision, obtained RSD values lower than 12.3% and 13.3%, respectively.

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P39. DEVELOPMENT OF AN ANALYTICAL METHOD FOR THE SIMULTANEOUS EXTRACTION AND CHROMATOGRAPHIC ANALYSIS OF TROPANE, PYRROLIZIDINE AND OPIUM ALKALOIDS IN COMMERCIAL BREAD SAMPLES

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Keywords: n*atural toxins, pyrrolizidine alkaloids, tropane alkaloids, opium alkaloids, UHPLC-MS/MS, solid phase extraction, food safety, bakery products, bread.*

Pyrrolizidine (PAs), tropane (TAs) and opium (OAs) alkaloids are natural toxins that have gained special interest in recent years because of their worrying effects that their intake may cause on the organism, from minor disorders (e.g., acute intoxications) to serious disorders (e.g., the onset of chronic diseases or even death). As a result, the Panel on Contaminants in the Food Chain (CONTAM) of the European Commission has issued several scientific opinions on the potential risks associated to the occurrence of these toxins in several foods and insists on further study of these compounds to help improve the actual estimation of the intake of these alkaloids by the population[1]. The foods more likely to be contaminated with these toxins are those of plant origin, such as cereals, seeds, herbs, spices, teas, herbal teas, honey, food supplements and their derived products. Accordingly, TAs have extensively been detected as contaminants in cereals and pseudocereals (e.g., millet, maize, buckwheat), which are often used for the preparation of gluten-free bakery products[1]. Indeed, nowadays there is a current trend among the population to eat gluten-free products. For this reason, R&D departments of food companies are making great efforts to improve the formulation of gluten-free products such as bread to achieve sensory and physicochemical properties like those containing gluten. As a result, it is increasingly common to find bakery gluten-free products with different spices (e.g., clove, aniseed, curry), aromatic herbs (e.g., oregano, rosemary, basil) and seeds (e.g., poppy seeds) in their ingredient formulation to make them more attractive and because they are sources of bioactive compounds. However, these may lead to the occurrence of PAs, TAs and OAs as contaminants in the same product. Therefore, it is suitable to develop analytical strategies that monitor the occurrence of these three types of alkaloids at the same time to ensure food safety. Accordingly, this work describes an analytical strategy to simultaneously determine the occurrence of 21 PAs, 2 TAs and 6 OAs in bread samples. For this purpose, the bread samples (previously milled to a fine powder) were first subjected to a solid-liquid extraction in acidified conditions, for which different extraction solvents (water with 1% HCl, methanol with 1% HCl and methanol:water (50:50) with 1% HCl) were evaluated. Afterwards, the sample extract was subjected to solid-phase extraction (SPE) for pre-concentration and clean-up. Strong-cation exchange (SXC) and mixed-mode cation exchange-reversed phase (MCX) polymeric cartridges were evaluated as sorbent materials. Better results were achieved with the MCX sorbent (recovery values 89-110%) than with SCX (recovery values 16-113%). The analytes were eluted under basic conditions, evaporated to dryness, and reconstituted in the mobile phase for their subsequent chromatographic analysis. During the process, different extraction parameters (e.g., extraction solvent, type of sorbent, amount of sorbent, elution volume, etc.) were evaluated and optimized. The chromatographic separation of the 29 alkaloids was achieved with a UHPLC system coupled to an ion trap tandem mass spectrometer detector operating in ESI positive mode and using a Luna Omega Polar C18 column (set at 30 ºC). The mobile phase was a mixture of water with 0.2% formic acid and methanol with 0.2% ammonia. The flow rate was maintained at 0.3 mL/min, the injection volume was 5 µL and the total analysis time was 15 min. Finally, the method was applied to the analysis of different commercial bread samples, showing that it is an interesting tool for the simultaneous determination of the three different alkaloid families.

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P40. DETERMINATION OF SILICON-CONTAINING COMPOUNDS BY GC-ICP-MS/MS IN PLASTIC-BASED PYROLYSIS OIL SAMPLES

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Keywords: s*ilicon compounds, plastic-based pyrolysis oil, GC-ICP-MS/MS.*

Pyrolysis has emerged as an interesting alternative for the recycling of plastic waste. However, during this process some unknown halogenated (such as silicon) products can be formed. The resulting pyrolysis oil can be used as a fuel or as polymers to produce new plastics. In the first case, halogenated products can lead to corrosion problems (SiO_2 formation) on the engines, and in the second case, they can damage the catalysts used along industrial production. Due to this problem, the presence of silicon in plastic pyrolysis oils needs to be rapidly detected and, if necessary, characterized.

In this communication, we present a GC-ICP-MS/MS approach adapted for silicon analysis in real pyrolysis plastic oils sample. This GC-ICP-MS/MS methodology affords both, speciation and total quantification analysis for real samples using a generic Si-containing standard after the optimization of a species-independent quantification method. The quantification of the silicon species was carried out by chromatography column and an internal standard, while total silicon quantification analysis was performed with a transfer line and the use of external calibration. The matrix effect of the sample was also evaluated using standard additions.

Two real pyrolysis oils from plastic samples were analyzed applying the abovementioned methodology, obtaining results that cover different silicon species and different ranges of concentration. Different columns, solvents for the sample and temperatures of injection were evaluated to obtain the best result. The concentration of silicon in the sample was compared with a reference value obtained by X-Ray Fluorescence (XRF) spectroscopy. Under the optimal conditions of the method a detection limit of less than 1 ppb was obtained.

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P41. SEPARATION OF ISOMERIC FORMS OF UROLITHIN CONJUGATES USING CHROMATOGRAPHIC TECHNIQUES. APPLICATION FOR METABOTYPE ASSIGNMENT IN URINE SAMPLES

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Keywords: *urolithins, isomers, chromatographic separation, analytical development, supercritical chromatography, ion pair chromatography.*

Microbiota metabolites, which seem to be responsible of health beneficial effects[1], are produced in humans after the intake of foods. Urolithins are class pf polyphenols metabolites that can be found in foods that contain their precursors: ellagitannins and ellagic acid. After their absorption, they are conjugated with glucuronic acid producing different regiosimeric isomers circulating in plasma and reaching the different tissues as glucuronide conjugates. Different metabotypes have been reported depending on the final urolithins produced: Urolithin A (Uro-A), isoUrolithin A (isoUro-A) and Urolithin B (Uro-B). These glucuronides could be produced in diverse quantities with difference in regional isomers in individuals due to enzyme polymorphins which is an analytical challenge. However, the lack of an appropriate methodology to separate these isomers hinders their analysis in biological samples. The current methods to date have the limitation of not being able to distinguish between several of glucuronide isomers. Thus, the present work presented novel methodologies to identify and quantify the different urolithins isomers with enough resolution. Two different chromatographic methods were developed and optimized by using supercritical fluid chromatography or ion pair chromatography. Five urolithins glucuronides were studied: Uro-A 3- and 8-glucuronide; isoUro-A 9-glucuronide and isoUro- A 3-glucuronide; and Uro-B glucuronide. For this purpose, the optimal chromatographic conditions for each method were selected by studying different stationary phases, mobile phase compositions, temperatures and/or pressures. The proposed methods were fully validated and applied to analyze these metabolites in urine samples from different volunteers belonging to different metabotypes. This work could represent a significant advance to improve metabotype assignment and their implications in human health.

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P42. DETERMINATION OF ENDOCRINE DISRUPTORS IN BEE PRODUCTS

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Keywords: *analytical methods*, *microplastics, additives, bee products, method development.*

Currently, there is a great concern about the relationship between diet and its influence on physiological and pathological processes. Hence, eating habits have evolved in recent years, and the demand of organic food has increased with special interest in which come from beehives, such as honey and bee pollen, due to its multiple beneficial properties[1]. However, these pollinators are exposed to several polluting sources from various sectors, such as microplastics (MPs) due to the plastic pollution global problem. MPs are defined as plastic particles whose size is between 5 mm and 1 µm, of high persistence and ubiquity. Likewise, due to their small size, MPs can be transferred through the trophic chain, thus causing toxic effects associated with the retention of environmental pollutants and the release of additives used to improve their properties[2]. These contaminants can be transported to the hive through foraging by bees and be transferred to the bee products, in addition to beekeeping practices or even the food containers used to their storage and distribution. Considering, the lack of regulation of these emerging contaminants as well as the associated risks, it is necessary to evaluate their presence in bee food products to guarantee the food safety of consumers and the pollinators too.

Therefore, the main objective of this study in the first instance was to evaluate the presence of microplastics in bee pollen and honey of different botanical origins through spectroscopic techniques such as attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR), µ-Raman and scanning electron microscope (SEM). In addition, it was developed an analytical method based on solid phase extraction (SPE) combined with high performance liquid chromatography coupled to tandem mass spectrometry to determine bisphenol A and 13 bisphenol analogues, which are classified as endocrine disruptors (EDCs). Several stationary phases were tested, and the effect of different chromatographic parameters (temperature, flow rate, type, and percentage of organic modifier) as well as those related to the detector (temperature of ionization source, capillary voltage, etc) were optimized. Finally, the proposed method was successfully applied to quantify the target EDCs in bee pollen and honey.

Acknowledgments
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P43. SIMPLE, RAPID AND HIGH-THROUGHPUT ANALYTICAL DETECTION PLATFORM FOR THE DETERMINATION OF TROPANE ALKALOIDS IN BEVERAGES

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Keywords: *mesostructured silicas, conducting polymers, screen printed electrodes, tropane alkaloids, beverages.*

Tropane alkaloids (TAs) are a group of toxic substances with hallucinogenic effects after consumption. Their presence in food of plant origin is due to contamination with tropane alkaloid-producing plant species, such as those belonging to the Brassicaceae, Solanaceae and Erythroxylaceae families[1]. According to the External Scientific Report published by EFSA in 2016 about the occurrence of tropane alkaloids in food, one or more TAs were detected in 70.2% of dry (herbal) tea out of 121 teas samples analysed[2]. Due to the potential risk to human health posed by the ingestion of food contaminated with tropane alkaloids, the development of rapid, simple, and environmentally friendly analytical methodologies to monitor their presence in food is of the utmost importance. In this respect, electroanalytical approaches can offer portability with fast and reliable detection while offering high sensitivity and selectivity towards the electroactive analytes. The use of screen-printed electrodes (SPE) provides disposable and affordable sensors due to their high reproducibility, high reliability, low cost, mass production, and in situ monitoring capability. SPEs have a flexible surface for modification, allowing them to be analysed for a variety of analytes and upgradingfor different purposes. In this regard, ordered mesostructured silicas (OMS) have attracted great interest in electrochemistry due to their excellent characteristics, such as their highly porous and regularly ordered structure and extremely high specific surface area, other important properties such as high thermal and mechanical stability, high adsorption capacity, and the ability to be functionalised[3]. These characteristics make them very interesting for use as SPE modifiers; however, thesematerials generally present low electronic conductivity, which can translate into a low sensor sensitivity. To overcome this situation, the main objective of this work was to design an analytical detection platform employing ordered mesostructured, printed polymers combining the merits of OMS and the excellent electronic properties of conductive polymers for the fast, simple and selective determination of TAs in beverages samples.

In this sense, various ordered mesostructured imprinted polymers were prepared using different proportions of SBA-15 as OMS base material, pyrrole or aniline as conducting monomers, and atropine sulfate as the analyte template. The resulting nanocomposites were characterized by powder X-ray diffraction, TEM, SEM, N2 adsorption, FT-IR spectrometry, ^{29}Si-NMR and ^{13}C-NMR spectrometry, elemental analysis, etc., in order to get the necessary information on structural, composition, and porosity parameters. Finally, the materials were applied as SPE modifiers for the electrochemical determination of TAs have been investigated. The effect of the different textural characteristics and electroactive properties of these materials have been studied by using $[Fe(CN)6]^{3-/4-}$ and TAs in a diffusional process, employing cyclic voltammetry and differential pulse voltammetry. Factors affecting the detection have been investigated, including electrode composition (medium and concentration of suspension), electrolysis and stripping media, and electrolysis time. The developed method will be validated in terms of linearity, selectivity, precision, and accuracy and will be applied forthe determination of TAs in beverages.

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P44. IDENTIFICATION OF EMERGING CONTAMINANTS ATTACHED TO MICROPLASTICS IN SEAFOOD AS A POTENTIAL SOURCE OF HUMAN EXPOSURE

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Keywords: *marine microplastic pollution, food safety, risk exposure assessment, high-resolutionmass spectrometry, emerging contaminants*

Microplastics are extremely persistent, ubiquitous and have the ability to accumulate emerging contaminants (ECs)[1]. Marine organisms ingest microplastics and the ECs are transferred through the food chain, and may potentially reach humans through the consumption of fish and shellfish[2]. The oceans and seas are the main food resource of humanity. Therefore, the establishment ofregulations is imperative for ensuring food safety for citizens, by monitoring the appropriate chemicals. In this study, a new suspect screening analytical methodology for the assessment of human exposure to microplastic pollutants through the identification of ECs in marine foods has been developed. For sample treatment has been proposed the use of SUPRAS, which represents a wholly new paradigm in processing because of the multiple opportunities they offer for improving process yields, selectivity, sustainability and economics. Supramolecular solvents (SUPRAS) are green nanostructured liquids spontaneously produced in colloidal suspensions of amphiphilic compounds by sequential self-assembly and coacervation. SUPRASs have outstanding properties for analyte extraction and sample cleanup, thus they can be tailored by proper design of the components and/orthe environment to remove major matrix components while effectively extract the target compounds[3]. High-resolution mass spectrometry (HRMS) was used for the detection and facilitates annotationand identification of ECs in marine foods. In addition, a dedicated data-analysis workflow for the annotation and identification of ECs in sea food samples was developed.

Since it may be a relationship between the distribution of microplastics throughout the water column and their intake, various edible marine species from different levels of marine water column were analysed. Individuals were gutted in order to search for microplastics in the digestive system. Microplastics found were submitted to a leaching test and the lixiviate was analysed by SUPRAS- HRMS. In the same way, muscle tissues were analysed according to the developed workflow for the identification of ECs.

ECs belonging to different chemical classes were identified in the tissue of marine organisms analysed. Thus, ECs related to plastics, such as the organophosphate flame retardants 2,3- dihydroxypropyl 2-(hexadecanoylamino)ethyl hydrogen phosphate and [(2R)-2-acetyloxy-3- hexadecoxypropyl] 2-(trimethylazaniumyl)ethyl phosphate, and the phthalate diisobutyl phthalate were detected, annotated and identified with a confident level 2 (Schymanski´s scale)[4]. The ECs identified with a level of confidence 3-1 (Schymanski´s scale) found in muscle tissue were crossed checked with the identified ECs in the lixiviate of microplastics found, in order to elucidate the potential relationships between microplastics and ECs presence in edible marine organisms. This study deepens the knowledge of the mechanisms of ECs migration and microplastics contribution to marine species contamination, assessing the potential risk of exposure to ECs through diet.

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P45. EVALUATION OF EXPOSURE TO EMERGING MYCOTOXINS THROUGH PORK URINE ANALYSIS

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Keywords: *enniatin, beauvericin, urine, biological sample, HPLC, mass spectrometry.*

Enniatins (ENNA, ENNA1, ENNB and ENNB1) and beauvericin (BEA) are mycotoxins of the genus *Fusarium*, which belong to the group of emerging mycotoxins, being generated in agricultural products under certain conditions of humidity and temperature and have been detected and quantified in several foods for human and animal consumption. In terms of toxicity, cytotoxic and apoptotic activities in animals have been reported in several studies. However, they are not regulated in food or feed due to the limited conclusive data on their toxicity according to the European Food Safety Authority (EFSA)[1,2]. Exposure to these mycotoxins has been studied in several biological fluids, with urine being one of the most frequently analysed samples due to its easy collection and availability. Furthermore, urine contains high levels of metabolites and provides information on metabolism of different parts of the body[3].

In this work, an efficient and environmentally friendly procedure is proposed for the determination of ENNs and BEA in pig urine samples in order to explore the occurrence of these mycotoxins and its derived metabolites. For this purpose, urine samples were subjected to twoconsecutive extraction steps: a salting out liquid-liquid extraction (SALLE) followed by a dispersive liquid-liquid microextraction (DLLME). Acetonitrile was used as extractant solvent in SALLE, and the collected supernatant after this step was then used in DLLME step. The samples were then analysed by high performance liquid chromatography coupled with tandem mass spectrometry using electrospray ionization (HPLC-ESI-MS/MS).

The proposed method was optimized and validated out in terms of linearity, limits of detection and quantification, precision, trueness, and matrix effect. Suitable linear dynamic ranges and low limits were achieved up to 0.01 ng mL^{-1} for quantification. To check the applicability of the method, 55 urine samples from pigs from nine different farms were analysed by the proposed methodology. As a result, all mycotoxins were detected in urine, with ENNB being the mycotoxin with the highest incidence and ENNB1 the lowest incidence. The concentration range of each mycotoxin in urine samples was 0.13- 311 ng mL^{-1} (ENNB), 0.75-21 ng mL^{-1} (BEA), 0.74-9.1 ng mL^{-1} (ENNB1), 0.44-11.1 ng mL^{-1} (ENNA1) and 0.54-24 ng mL^{-1} (ENNA).

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P46. ENVIRONMENTAL CHARACTERIZATION OF METALLOPHORES AND THEIR POTENTIAL MICROBIAL PRODUCERS IN PEATLANDS

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Keywords: e*nvironment, mass spectrometry, liquid chromatography, metallophores, peatlands.*

To scavenge soluble essential metals (e.g., Fe, Cu, Zn, etc.) from the environment, microorganisms produce and secrete low molecular weight biogenic ligands called metallophores. Metallophores are biosynthesized by bacteria, fungi, and plants and excreted under metal starvation, i.e., when intracellular metal concentrations drop below a certain threshold required for the functionality of biochemical processes in the cell[1].

The microorganism growth dependence of metal availability reflects the relevance of metallophores involvement in microbiome interactions or growth mechanisms, among others. In microorganisms, the study and characterization of metallophores, mainly siderophores (iron chelators), are generally based on the culture of bacterial strains and use metal-encoded isotope profiling approaches. This consists of the incubation of the sample with a certain iron isotopic tag that will produce a specific isotope pattern fingerprint to be detected by high-resolution mass spectrometry (MS)[2].

However, these approaches are strongly conditioned by the low concentration of metallophores, especially in alkaline environments where metal availability is low[3], the complexity of the environmental matrix, which affects the efficiency of complexation with the isotopic marker, or the limited number of cultivable bacterial strains. As a result, only a fraction of the potential pool of metallophores is revealed[4]. Therefore, we aimed to develop an analytical platform to unveil and characterize the metallophores pool directly from any environmental sample.

We have developed an analytical methodology to identified metallophores through the detection of MS natural isotope patterns characteristic of metal complexes together with mass-based database searches. To do so, we combined 2D separation using on-line solid phase extraction together with RP-HPLC, resulting in x100 enrichment and separation of metallophores directly in environmental samples in less than 20 minutes of analysis, followed by high-resolution MS analysis (< 3ppm error). That way, we were able to identify over 50 complex and non-complexed metallophores in environmental samples in concentration levels as low as fmol/g. Furthermore, a taxonomic-based inference method was implemented based on literature and public databases (antiSMASH database version 3.0) searches allowing to associate over 40% of the identified bacterial metallophores with potential producers.

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