

**Atti del XXIX Congresso  
della Divisione di Chimica Analitica  
della Società Chimica Italiana**

Milazzo (Messina)

11-15 Settembre 2022

<https://www.analitica2022.chim.it>



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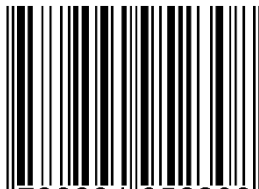
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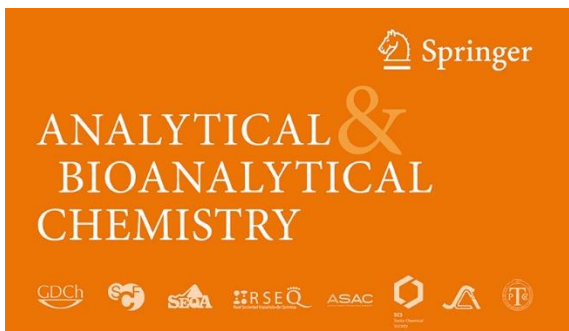
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### PRESENTAZIONE

Il Congresso, che la Divisione di Chimica Analitica della Società Chimica Italiana organizza annualmente, vuole essere un punto di incontro e di confronto per tutti coloro che svolgono la propria attività nella ricerca chimico analitica.

Gli organizzatori cercheranno di coprire nel modo migliore i diversi aspetti della chimica analitica, dai settori tradizionali, a quelli più innovativi.

In questa edizione sarà data particolare enfasi al tema *La Chimica Analitica per un futuro verde e sostenibile*.

I partecipanti sono cordialmente invitati a presentare i risultati della propria attività di ricerca con comunicazioni orali o poster.

I principali argomenti di discussione previsti sono i seguenti:

- Alimenti e Nutraceutici
- Ambiente e Beni Culturali
- Bioanalitica e Omics
- Chemiometria e Qualità del Dato
- Chimica Analitica Forense
- Elettroanalitica
- Equilibri in Soluzione e Speciazione
- Green Chemistry
- Sensori e Biosensori
- Spettrometria di Massa
- Spettroscopia Analitica
- Scienza delle Separazioni
- Tossicologia e Salute Umana

L'organizzazione è curata dai gruppi di Chimica Analitica dell'Università degli Studi di Messina.



# Programma

## Domenica 11 settembre 2022

17.00 – 21.00 Registrazione dei partecipanti (Sala: Duomo)

19.00 – 21.00 Cocktail di benvenuto (Sala: Duomo)

## Lunedì 12 settembre 2022

Sala: Duomo

Dalle 08.30

Registrazione dei partecipanti

09.00 – 09.30

Apertura del Congresso

09.30 – 10.15

Conferenza Plenaria **PL1** – Moderatore: Prof. Claudio Minero

**Prof. Damià Barceló** (IDAEA-CSIC, ICRA – Catalan Institute for water research, Spain) *MICROPLASTICS IN THE AQUATIC ENVIRONMENT: GREEN ANALYTICAL PROTOCOLS, RISKS AND SUSTAINABLE SOLUTIONS.*

10.15 – 10.35

Conferenza Vincitore del Premio Giovane Ricercatore **GR1**. Dott. Paolo Bollella, Università degli Studi di Bari Aldo Moro. *ENZYME-BASED AMPEROMETRIC BIOSENSORS: 60 YEARS LATER... QUO VADIS?*

10.40 – 11.10

Coffee Break (Sala: Duomo)

Sala: Duomo

Sessione Parallela: **Ambiente e Beni Culturali 1 (AMB\_1)**

Moderatori: Proff. Claudio Minero e Gianpiero Adami

11.10 – 11.30 AMB-KN1 *EFFECTIVE DEGRADATION OF IBUPROFEN THROUGH AN ELECTRO-FENTON PROCESS IN THE PRESENCE OF ZERO-VALENT IRON*

M. Minella, L. Martone, C. Minero, F. Sordello, D. Vione

Università degli Studi di Torino

11.30 – 11.45 AMB-OR01 *CONTROLLED PERIODIC ILLUMINATION AS VALUABLE STRATEGY FOR IMPROVING HER OVER METAL-SEMICONDUCTOR NANOPARTICLES AND STUDYING THE PHOTOCATALYTIC PROCESSES*

F. Pellegrino, M. Prozzi, F. Sordello, C. Minero, V. D. Hodoroaba, J. Radnik, V. Maurino

Università degli Studi di Torino

11.45 – 12.00 AMB-OR02 *MICROPLASTIC IN DRINKING WATER: A NEW ANALYTICAL CHALLENGE*

E. Brancaleone, V. Fuscoletti, L. Lucentini, D. Mattei, G. Favero, A. Frugis, V. Gioia, M. Lazzazzara

Istituto Superiore di Sanità

- 12.00 – 12.15 AMB-OR03 *BIOACCUMULATION AND ECOTOXICOLOGICAL EVALUATION OF BISPHENOL ANALOGUES IN RUDITAPES PHILIPPINARUM: FROM TARGETED ANALYSIS TO NON-TARGETED FINDINGS*

M. Roverso, C. Pellegrini, Y. Ben Hadj, J. Fabrello, V. Matozzo, S. Bogialli

Università degli Studi di Padova

- 12.15 – 12.30 AMB-OR04 *OPTIMIZATION OF A NEW MULTI-REAGENT PROCEDURE FOR QUANTITATIVE MUSSEL DIGESTION IN MICROPLASTIC ANALYSIS*

S. Fraissinet, A. Pennetta, S. Rossi, G. E. De Benedetto, C. Malitesta

Università degli Studi del Salento (Lecce)

- 12.30 – 12.45 AMB-OR05 *OPTIMIZATION OF AN EASY, EFFECTIVE AND ROBUST PROTOCOL FOR THE EXTRACTION AND QUANTITATION OF MICROPLASTICS IN SOIL MATRICES*

L. Rivoira, A. E. Carioli, M. Castiglioni, P. Bracco, E. Padoan, F. Ajmone Marsan, G. Cecconi, M. C. Bruzzoniti

Università degli Studi di Torino

- 12.45 – 13.00 AMB-OR06 *PROGRESS ON SOURCE ASSESSMENT OF POLLUTANTS REACHING THE POLAR REGIONS BY ELEMENTAL AND ISOTOPIIC ANALYSIS*

F. Ardini, M.A. Vecchio, S. Bertinetti, M. Grotti

Università degli Studi di Genova

## **Sala: Convento 2**

### **Sessione Parallela: Scienza delle Separazioni + Spettrometria di Massa 1 (SEPMS\_1)**

Moderatori: Proff. Alberto Cavazzini e Tommaso Cataldi

- 11.10 – 11.30 SEPMS-KN01 *ENANTIOSELECTIVE LIQUID CHROMATOGRAPHY ANALYSIS OF AMINO ACIDS UNDER ECO-FRIENDLY CONDITIONS*

I. Varfaj, F. Ianni, A. Carotti, R. Sardella

Università degli Studi di Perugia

- 11.30 – 11.45 SEPMS-OR01 *FAST UNTARGETED LIPIDOMICS BY ULTRA-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY-TRAPPED ION MOBILITY MASS SPECTROMETRY: A PROOF OF CONCEPT*

E. Sommella, F. Merciai, P. Campiglia

Università degli Studi di Salerno

- 11.45 – 12.00 SEPMS-OR02 *HUMAN CHEMOSIGNALS ELICITED FROM EMOTIONAL STIMULATION: AN INVESTIGATION BY COMPREHENSIVE GCXGC HIGH RESOLUTION MS*

T. Bruderer, M. Ripszam, A. Baldini, A. L. Callara, D. Biagini, S. Ghimenti, T. Lomonaco, A. Greco, E. P. Scilingo, F. Di Francesco

Università degli Studi di Pisa

- 12.00 – 12.15 SEPMS-OR03 *DEVELOPMENT OF A RPWAX×RPLC-HRMS PLATFORM FOR THE NON-TARGETED IDENTIFICATION OF PFASs IN AQUEOUS FILM-FORMING FOAMS*

L. Renai, A. F. G. Gargano, S. Samanipour, P.J. Schoenmakers, M. Del Bubba

Università degli Studi di Firenze

- 12.15 – 12.30 SEPMS-OR04 *TARGETED AND UNTARGETED CHARACTERIZATION OF UNDERIVATIZED POLICOSANOLS IN HEMP INFLORESCENCE BY LIQUID CHROMATOGRAPHY-HIGH RESOLUTION MASS SPECTROMETRY*  
S.E. Aita, A.L. Capriotti, A. Laganà, L. Mondello  
 Università degli Studi di Roma La Sapienza
- 12.30 – 12.45 SEPMS-OR05 *COUPLING OF LIQUID CHROMATOGRAPHY TO NEGATIVE CHEMICAL IONIZATION MASS SPECTROMETRY: A NEW ANALYTICAL APPROACH FOR THE ANALYSIS OF PER- AND POLYFLUOROALKYL SUBSTANCES*  
M. Dutt, A. Arigò, P. Palma, G. Famiglioni, A. Cappiello  
 Università degli Studi di Urbino Carlo Bo
- 12.45 – 13.00 SEPMS-OR06 *IDENTIFICATION OF PHOTODEGRADATION PRODUCTS OF ESCITALOPRAM IN SURFACE WATER BY HPLC-MS/MS*  
F. Gosetti, L. Valenzisi, V. Termopoli, V. Consonni, D. Ballabio, R. Todeschini, M. Orlandi  
 Università degli Studi di Milano-Bicocca

#### Sala: Convento 5

Sessione Parallela: **Bioanalitica e Omics 1 (BIOAN\_1)**

Moderatori: Proff. Aldo Roda e Anna Laura Capriotti

- 11.10 – 11.30 BIOAN-KN01 *HIGH SENSITIVITY vs SIMPLICITY: DETECTION STRATEGIES FOR LATERAL FLOW ASSAY*  
L. Anfossi, F. Di Nardo, S. Cavalera, C. Baggiani  
 Università degli Studi di Torino
- 11.30 – 11.45 BIOAN-OR01 *FAST LC-MS/MS SCREENING METHOD FOR THE EVALUATION OF DRUGS, ILLICIT DRUGS, AND OTHER COMPOUNDS IN UNCONVENTIONAL BIOLOGICAL MATRICES*  
M. Locatelli, A. Tartaglia, E. Rosato, G.M. Merone, E. Bassotti, C. D'Ovidio, U. de Grazia, K.G. Furton, H.I. Ulusoy, A. Kabir, F. Savini  
 Università degli Studi di Chieti-Pescara G. D'Annunzio
- 11.45 – 12.00 BIOAN-OR02 *A NOVEL AZA-PATERNÒ-BÜCHI REACTION FOR PINPOINTING CARBON-CARBON DOUBLE BONDS IN LIPIDS BY HIGHER COLLISIONAL DISSOCIATION MASS SPECTROMETRY*  
A. Cerrato, A. L. Capriotti  
 Università degli Studi di Roma La Sapienza
- 12.00 – 12.15 BIOAN-OR03 *AUTOMATIC AND MINIATURIZED EXTRACTION COUPLED WITH ON LINE UHPLC-MS/MS FOR THE ANALYSIS OF VITAMIN D METABOLITES AND INTACT LIPIDS FROM DRIED BLOOD SPOT*  
D. Donnarumma, G. Micalizzi, F. Rigano, L. Mondello  
 Università degli Studi di Messina
- 12.15 – 12.30 BIOAN-OR04 *PROGRAMMABLE BIOSENSING INTERFACES BASED ON DYNAMIC DNA SYSTEMS INTEGRATED WITH NANOSTRUCTURED ELECTRODES*  
 S. Fortunati, M. Giannetto, A. Porchetta, M. Careri, A. Bertucci  
 Università degli Studi di Parma
- 12.30 – 12.45 BIOAN-OR05 *RABBIT IgG-IMPRINTED NANOMIPS BY SOLID PHASE SYNTHESIS AS MIMIC OF PROTEIN A: EFFECT OF CROSS-LINKER ON AFFINITY AND SELECTIVITY*  
 L. Anfossi, C. Baggiani, S. Cavalera, M. Chiarello, F. Di Nardo, T. Serra, V. Testa  
 Università degli Studi di Torino

- 12.45 – 13.00 *BIOAN-OR06 FAST DETERMINATION OF ENDOCANNABINOIDS AND RELATED COMPOUNDS BY MEANS OF UHPLC-MS/MS IN BIOLOGICAL MATRICES*  
F. Fanti, C. Montesano, F. Vincenti, G. Imparato, M. Maccarrone, M. Sergi, D. Compagnone  
 Università degli Studi di Teramo
- 13.00 – 14.00 Lunch (libero)
- 14.00 – 15.00 Sessione POSTER: EQ, SPETTRO, BIOANAL, AMB (Sala: Convento 3)

**Sala: Duomo**

Sessione Parallela: **Scienza delle Separazioni + Spettrometria di Massa 2 (SEPMS\_2)**

Moderatori: Proff. Maria Careri e Achille Cappiello

- 15.00 – 15.20 *SEPMS-KN02 HIGH RESOLUTION MASS SPECTROMETRY: ANALYTICAL CHALLENGES AND NEW OPPORTUNITIES*  
F. Bianchi, N. Riboni, M. Mattarozzi, M. Meleti, M. Peracchia, M. Careri  
 Università degli Studi di Parma
- 15.20 – 15.35 *SEPMS-OR07 EXPLORING THE RETENTION BEHAVIOR OF CANNABINOIDS UNDER NORMAL- AND REVERSED-PHASE CONDITIONS ON IMMOBILIZED POLYSACCHARIDE-BASED CHIRAL STATIONARY PHASES*  
S. Felletti, C. De Luca, W. Umstead, P. Franco, M. Catani, A. Cavazzini  
 Università degli Studi di Ferrara
- 15.35 – 15.50 *SEPMS-OR08 DEVELOPMENT AND VALIDATION OF A LIQUID CHROMATOGRAPHY – MASS SPECTROMETRY METHOD FOR MULTIRESIDUE ANALYSIS IN MUSSELS OF THE ADRIATIC SEA*  
N. Interino, R. Comito, E. Porru, P. Simoni, S. Franzellitti, M. Candela, A. Roda, J. Fiori  
 Università degli Studi di Bologna
- 15.50 – 16.05 *SEPMS-OR09 DEVELOPMENT AND VALIDATION OF MULTI-CLASSES OF PESTICIDES IN CORN-BASED FOOD USING COMPREHENSIVE TWO-DIMENSIONAL LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY (LC×LC-MS/MS)*  
K. Arena, L. Martin-Pozo, Y. Oulad El Majdoud, F. Cacciola, P. Dugo, L. Mondello  
 Università degli Studi di Messina
- 16.05 – 16.20 *SEPMS-OR10 SUSPECT SCREENING APPROACH FOR THE IDENTIFICATION OF SULFONAMIDES TRANSFORMATION PRODUCTS IN WATER SAMPLES*  
B. Giannelli Moneta, C. Cavaliere  
 Università degli Studi di Roma La Sapienza
- 16.20 – 16.35 *SEPMS-OR11 EFFECTS OF MULTIFUNCTIONAL BUFFERING AGENTS AND ADDITIVES OF THE BACKGROUND ELECTROLYTE SOLUTION ON ELECTROOSMOTIC FLOW AND MIGRATION BEHAVIOUR OF THE ANALYTES IN CAPILLARY ZONE ELECTROPHORESIS*  
D. Corradini  
 CNR Roma
- 16.35 – 17.00 Coffee Break (Sala: Duomo)

- 17.00 – 17.15 SEPMS-OR12 *THE COMBINATION OF RPLC-ESI-FTMS/MS AND M-CPBA EPOXIDATION FOR DOUBLE BOND LOCATION AND GEOMETRY ASSIGNMENT IN UNSATURATED FREE AND TOTAL FATTY ACIDS*  
D. Coniglio, C.D. Calvano, I. Losito, T.R.I. Cataldi  
 Università degli Studi di Bari Aldo Moro
- 17.15 – 17.30 SEPMS-OR13 *AMBIENT MASS SPECTROMETRY AND SWABBING TEST STRATEGIES FOR INNOVATIVE HIGH-THROUGHPUT ANALYSIS OF ALLERGENIC PROTEINS*  
M. Mattarozzi, L. Toma, S. Gentili, E. Moyano, M. Careri  
 Università degli Studi di Parma
- 17.30 – 17.45 SEPMS-OR14 *HIGH-RESOLUTION MS DETECTION OF PESTICIDES USING THE DIRECT COUPLING OF A SPME FIBER WITH A LIQUID- EI INTERFACE*  
G. Grasselli, N. Marittimo, G. Famiglini, P. Palma, A. Cappiello  
 Università degli Studi di Urbino Carlo Bo
- 17.45 – 18.00 SEPMS-OR15 *INFLUENCE OF SAMPLING ADSORBENTS FOR TRACKING VOLATILE METABOLITES FROM IN VITRO AND EX VIVO BIOLOGICAL SAMPLES*  
 S. Malcangi, M. Romagnoli, M. Beccaria, A. Cavazzini, F.A. Franchina  
 Università degli Studi di Ferrara

**Sala: Convento 2**

Sessione Parallela **Equilibri in Soluzione e Speciazione (EQ)**

Moderatori: Proff. Giuseppe Arena e Pier Giuseppe Daniele

- 15.00 – 15.20 EQ-KN01 *THE ROLE, THE BEHAVIOUR AND THE SPECIATION OF THE METAL CATIONS IN MIMETIC SYSTEMS*  
R.M. Cigala, F. Crea, C. De Stefano  
 Università degli Studi di Messina
- 15.20 – 15.35 EQ-OR01 *FIRST-ROW TRANSITION METAL CATIONS COMPLEXES OF LUTEOLIN: STABILITY CONSTANTS AND STRUCTURAL CHARACTERIZATION IN AQUEOUS MEDIA*  
 L. Malacaria, E. Furia  
 Università degli Studi della Calabria
- 15.35 – 15.50 EQ-OR02 *ON THE ACID-BASE AND METAL IONS ADSORPTIVE PROPERTIES OF COLLOIDAL CARBON-BASED NANOPARTICLES*  
C. Manfredi, M. Trifuoggi, V. Gargiulo, M. Alfé  
 Università degli Studi di Napoli Federico II
- 15.50 – 16.05 EQ-OR03 *A FAMILY OF KOJIC ACID DERIVATIVES FOR THE REMEDIATION OF Pb<sup>2+</sup> AND Cd<sup>2+</sup>: COMPLEX FORMATION EQUILIBRIA STUDIES*  
R. Cappai, A. Fantasia, G. Crisponi, V. M. Nurchi  
 Università degli Studi di Cagliari
- 16.05 – 16.20 EQ-OR04 *PYES – AN OPEN SOURCE SOFTWARE FOR THE COMPUTATION OF IN SOLUTION AND PRECIPITATION EQUILIBRIA*  
L. Castellino, E. Alladio, S. Bertinetti, G. Lando, C. De Stefano, S. Gama, S. Berto, D. Milea  
 Università degli Studi di Torino
- 16.20 – 16.35 EQ-OR05 *HIGHLY SELECTIVE DETERMINATION OF METHYLMERCURY IN BIOLOGICAL SAMPLES: AN ICP-MS METHOD BASED ON THE ON-LINE BLOCKING OF INORGANIC MERCURY*

D. Spanu, S. Recchia, D. Monticelli

Università degli Studi dell'Insubria

16.35 – 17.00 Coffee Break (Patio Convento)

17.00 – 17.15 EQ-OR06 *THERMODYNAMIC BEHAVIOR AND SENSING PROPERTIES OF TWO CARNOSINE-BASED DERIVATIVES*

C. Abate, A. Fragoso, O. Giuffrè, A. Mazzaglia, A. Piperno, C. Foti

Università degli Studi di Messina

17.15 – 17.30 EQ-OR07 *CHELATING PROPERTIES OF THE FUNGAL METABOLITE HARZIANIC ACID TOWARD TOXIC HEAVY DIPOSITIVE CATIONS ( $Cd^{2+}$ ,  $Co^{2+}$ ,  $Ni^{2+}$ ,  $Pb^{2+}$ )*

G. De Tommaso, M. M. Salvatore, A. Staropoli, F. Vinale, R. Nicoletti, M. Della Greca, F. Salvatore, M. Lorito, M. Iuliano, and A. Andolfi

Università degli Studi di Napoli Federico II

17.30 – 17.45 EQ-OR08 *THE IMPACT OF METAL COORDINATION ON CALCITERMIN ANTIMICROBIAL PROPERTIES*

D. Bellotti, S. Leveraro, E. Dzień, M. Rowińska-Żyrek, M. Remelli

Università degli Studi di Ferrara

17.45 – 18.00 EQ-OR09 *METAL-COORDINATED ASSEMBLIES BASED ON QUERCETIN: SOLUTION EQUILIBRIA ARE CRUCIAL FOR DEVELOPING pH-RESPONSIVE DRUG DELIVERY SYSTEMS*

G.D.G. Santonoceta, C. Sgarlata

Università degli Studi di Catania

#### **Sala: Convento 5**

#### **Sessione Parallela: Spettroscopia Analitica (SPETTRO)**

Moderatori: Dott.ssa Emilia Bramanti e Prof.ssa Antonella Rossi

15.00 – 15.20 SPETTRO-KN01 *DO WE REALLY NEED NANOMATERIALS IN THE FIGHTING AGAINST SARS-CoV-2? THE OPINION OF AN ANALYTICAL SPECTROSCOPIST*

M.C. Sportelli, M. Izzi, A. Sallustio, D. Loconsole, R.A. Picca, R. Felici, M. Chironna, N. Cioffi  
Università degli Studi di Bari Aldo Moro

15.20 – 15.35 SPETTRO-OR01 *INFRARED SPECTROSCOPY INVESTIGATION OF THE SECONDARY STRUCTURE OF GELATIN CONTAINED IN DIFFERENT GELATIN/WASTE KERATIN-BASED BIOMATERIALS*

E. Pulidori, S. Micalizzi, F. Montemurro, C. De Maria, G. Vozzi, M. R. Tinè, C. Duce, E. Bramanti

Università degli Studi di Pisa

15.35 – 15.50 SPETTRO-OR02 *AN X-RAY PHOTOELECTRON SPECTROSCOPY (XPS) STUDY OF FRAGMENTATION PROCESS OF PLASTIC MATERIALS*

T. Di Giulio, S. Fraissinet, A. Pennetta, G. E. De Benedetto, E. Mazzotta, C. Malitesta  
Università degli Studi del Salento (Lecce)

15.50 – 16.05 SPETTRO-OR03 *ANALYTICAL SPECTROSCOPY INVESTIGATIONS IN THE GALVANIC INDUSTRY TO STUDY INTERMETALLIC DIFFUSION*

M. Verrucchi, W. Giurlani, F. Biffoli, E. Mariani, G. Pappaianni, M. Vizza, M. Innocenti

Università degli Studi di Firenze

16.05 – 16.20 SPETTRO-OR04 *EXPLORING PLASMONIC-BASED IMAGING SENSOR FOR ULTRASENSITIVE MOLECULAR DIAGNOSTICS: THE ROAD TO LIQUID BIOPSY*

R. D'Agata, N. Bellassai, G. Spoto

Università degli Studi di Catania

1620 – 16.35 SPETTRO-OR05 *POLYMER COMPOSITION ANALYSIS OF PLASTIC DEBRIS INGESTED BY LOGGERHEAD TURTLES (CARETTA CARETTA) IN SOUTHERN TYRRHENIAN SEA THROUGH ATR-FTIR SPECTROSCOPY*

C. Bruno, M.F. Blasi, D. Mattei, L. Martellone, E. Brancaleone, S. Savoca, G. Favero

Università degli Studi di Roma La Sapienza

16.35 – 17.00 Coffee Break (Patio Convento)

17.00 – 17.15 SPETTRO-OR06 *EXPLOITING P-X-RAY FLUORESCENCE AS METHOD FOR THE ANALYSIS OF ANCIENT POTTERY*

M. Fantauzzi, A. Rossi

Università degli Studi di Cagliari

17.15 – 17.30 SPETTRO-OR07 *COATINGS THICKNESS DETERMINATION USING SEM AND ENERGY DISPERSIVE X-RAY SPECTROSCOPIES WITH A STANDARDLESS APPROACH*

R. Emanuele, W. Giurlani, F. Pizzetti, S. M. Martinuzzi, M. Vizza, M. Bonechi, M. Innocenti

Università degli Studi di Firenze

17.30 – 17.45 SPETTRO-OR08 *ANALYTICAL CHARACTERIZATION AND ANTIMICROBIAL ACTIVITY OF GREEN ZEOLITE-THYMOL COMPOSITES*

S. Cometa, L. Pinto, F. Busto, M.A. Bonifacio, A. Bellissimo, A. Petrella, N. De Vietro, G. Iannaccone, F. Baruzzi, E. De Giglio

Università degli Studi di Bari Aldo Moro

17.45 – 18.00 SPETTRO-OR09 *THE EFFECT OF THE ARTIFICIAL SALIVA COMPOSITION FOR CORROSION STUDIES: EVIDENCE FROM XPS ANALYSES.*

D. Biggio, M. Fantauzzi, B. Elsener, A. Rossi

Università degli Studi di Cagliari

Sala: Duomo

18.30 – 20.00 Assemblea Plenaria della Divisione (Consegna Premi e Medaglie)

## **Martedì 13 Settembre 2022**

Sala: Duomo

09.00 – 09.45 Conferenza Plenaria PL2 – Moderatore: Prof. Giuseppe Spoto

Prof. Wolfgang Knoll (AIT Austrian Institute of Technology, Vienna, and Danube Private University, Krems, Austria)

*APTAMERS – NEW ASPECTS OF THEIR USE IN ELECTRONIC SENSING*

**Sala: Duomo**

Sessione Parallela: **Ambiente e Beni Culturali 2 (AMB\_2)**

Moderatori: Proff. Francesca Modugno e Antonio Proto



- 09.55 – 10.15 **AMB-KN02** *A MULTI-ANALYTICAL APPROACH BASED ON MALDI-MS AND SPECTROSCOPIC TECHNIQUES FOR THE IDENTIFICATION OF SPRAY PAINTS USED IN URBAN ARTWORKS*  
C.D. Calvano, E.C.L. Rigante, F. Modugno, D. Scalarone, R.A. Picca, L. Sabbatini, T. R. I. Cataldi  
 Università degli Studi di Bari Aldo Moro
- 10.15 – 10.30 **AMB-OR07** *THE BURIED OPIUM: ANALYTICAL DETERMINATION OF ALKALOIDS IN ANCIENT REMAINS*  
 F. Vincenti, A. Ciccola, M. Pallotta, I. Serafini, L. Leone, I. M. Muntoni, G. Favero, C. Montesano, R. Curini, M. Sergi  
 Università degli Studi di Roma La Sapienza
- 10.30 – 10.45 **AMB-OR08** *DEVELOPMENT OF ANALYTICAL PROTOCOLS TO ASSESS THE PERFORMANCE OF NEW GREEN METHODS FOR RESTORATION*  
S. Prati, F. Ramacciotti, K. Burcu, G. Sciutto G, M.L. Focarete ML, C. Gualandi, R. Mazzeo  
 Università degli Studi di Bologna
- 10.45 – 11.15 Coffee Break (Sala: Duomo)**
- 11.15 – 11.30 **AMB-OR09** *CHARACTERIZATION OF IN-LAB AGED LIPIDS BY USING GAS AND LIQUID CHROMATOGRAPHY APPROACHES COUPLED TO MASS SPECTROMETRY*  
V. Chiaia, G. Micalizzi, D. Donnarumma, A. Irto, C. Bretti, P. Cardiano, P. Q. Tranchida, L. Mondello  
 Università degli Studi di Messina
- 11.30 – 11.45 **AMB-OR10** *STABLE ISOTOPE RATIO OF CARBON, NITROGEN, OXYGEN AND SULFUR: APPLICATIONS IN CULTURAL HERITAGE AND NEW ANALYTICAL METHODOLOGIES*  
M. Ricciardi, C. Pironti, A. Faggiano, L. Bontempo, F. Camin, O. Motta, A. Proto  
 Università degli Studi di Salerno
- 11.45 – 12.00 **AMB-OR11** *POTENTIALLY HARMFUL ORGANIC COMPOUNDS LEACHED OUT FROM MICROPLASTICS: EXTRACTION METHODS AND DETECTION*  
G. Biale, J. La Nasa, M. Mattonai, A. Corti, V. Castelvetro, F. Modugno  
 Università degli Studi di Pisa
- 12.00 – 12.15 **AMB-OR12** *CHEMICAL SPECIATION OF ATMOSPHERIC PM FROM POLAR REGIONS*  
S. Bertinetti, S. Berto, M. Malandrino, D. Vione, D. Fabbri, E. Conca, M. Marafante, M. Rabbia, G. Guidi, O. Abollino, A. Annibaldi, C. Truzzi, S. Illuminati  
 Università degli Studi di Torino
- 12.15 – 12.30 **AMB-OR13** *BERGAMOT PASTAZZO AS SUSTAINABLE PLATFORM FOR Cd<sup>2+</sup> REMOVAL FROM AQUEOUS SOLUTION*  
A. Irto, O. Gómez-Laserna, G. Lando, C. De Stefano, P. Cardiano  
 Università degli Studi di Messina
- 12.30 – 12.45 **AMB-OR14** *PLASTICS IN THE MEDITERRANEAN SEA: INVESTIGATION OF THE PRESENCE IN TWO MARINE SPECIES AND POTENTIALLY CORRELATED ADVERSE EFFECTS*  
T. Chenet, A. Mancina, G. Bono, A. Baldi, A. Cavazzini, L. Pasti  
 Università degli Studi di Ferrara
- 12.45 – 13.00 **AMB-OR15** *DISINFECTION BY-PRODUCTS AND ECOTOXIC RISK ASSOCIATED WITH HYPOCHLORITE TREATMENT OF IRBESARTAN*  
A. Medici, A. Siciliano, M. Guida, G. Libralato, L. Saviano, G. Luongo, L. Previtiera, G. Di Fabio, A. Zarrelli  
 Università degli Studi di Napoli Federico II

**Sala: Convento 2**

Sessione Parallela: **Bioanalitica e Omics 2 (BIOAN\_2)**

Moderatori: Proff. Claudio Baggiani e Aldo Laganà

09.55 – 10.15 **BIOAN-KN02** *THE “CELL CHROMATOGRAPH”*

B. Roda, A. Zattoni, V. Marassi, S. Zia, P. Reschiglian

Università degli Studi di Bologna

10.15 – 10.30 **BIOAN-OR07** *ANALYTICAL WORKFLOW FOR SULFOPEPTIDE ENRICHMENT AND SEQUENCE ANALYSIS WITH SITE LOCALIZATION*

C.M. Montone, A.L. Capriotti

Università degli Studi di Roma La Sapienza

10.30 – 10.45 **BIOAN-OR08** *UNTARGETED LIPIDOMICS TO EVALUATE THE EFFECTS OF X-RAY IRRADIATION ON MOZZARELLA CHEESE*

A. Mentana, M. Campaniello, R. Zianni, M. Tomaiuolo, M. Iammarino, V. Nardelli

IZSPB Foggia

**10.45 – 11.15 Coffee Break (Patio Convento)**

11.15 – 11.30 **BIOAN-OR09** *PHYTOCANNABINOMICS: A TOOL FOR CANNABIS CHEMOVAR DIFFERENTIATION AND COMPOUND DISCOVERY*

C. Citti, G. Cannazza, A. L. Capriotti, F. Marini, A. Laganà

CNR Lecce

11.30 – 11.45 **BIOAN-OR10** *MASS SPECTROMETRY-BASED ASSAY FOR THE DETERMINATION OF NATRIURETIC PEPTIDES IN PLASMA SAMPLES*

A. Lenzi, M. De Cristofaro, D. Biagini, S. Ghimentì, F. Di Francesco, T. Lomonaco

Università degli Studi di Pisa

11.45 – 12.00 **BIOAN-OR11** *MALDI IMAGING-BASED SPATIAL-OMICS HIGHLIGHTS SPECIFIC METABOLITE AND LIPID SIGNATURES OF PAROTID TUMOR*

E. Salviati, E. Sommella, V. Caponigro, P. Campiglia

Università degli Studi di Salerno

12.00 – 12.15 **BIOAN-OR12** *SYNTHESIS OF  $Fe_3O_4$ -ACTIVATED CARBON FROM WASTEPAPER HANDKERCHIEFS FOR DISPERSIVE MAGNETIC SOLID-PHASE EXTRACTION OF NON-STEROIDAL ANTI-INFLAMMATORY DRUGS NSAIDS AND THEIR UHPLC-PDA DETERMINATION IN HUMAN PLASMA*

V. Ferrone, P. Bruni, S. Ferrari, G. Carlucci

Università degli Studi di Chieti-Pescara G. D'Annunzio

12.15 – 12.30 **BIOAN-OR13** *USE OF ONLINE BUFFER EXCHANGE COUPLED TO NATIVE MASS SPECTROMETRY TO ELUCIDATE SUBSTRATE-BINDING AFFINITY OF FRLB, A BACTERIAL DEGLYCASE*

A. Di Capua, S. Kovvali, V. Gopalan, V.H. Wysocki

Università degli Studi della Basilicata

12.30 – 12.45 **BIOAN-OR14** *A NUTRIMETABOLOMICS APPROACH TO UNDERSTAND PROTECTION FROM SARS-COV-2 INFECTION*

E. Barberis, E. Amede, P.P. Sainaghi, E. Marengo, M. Manfredi

Università degli Studi del Piemonte Orientale (Alessandria)

12.45 – 13.00 **BIOAN-OR15** *NATIVE FFF-CHARACTERIZATION AND QC PROFILING OF HUMAN AMNIOTIC STEM CELL VESICULAR FRACTIONS FOR SECRETOME-BASED THERAPY*

V. Marassi, S. Giordani, T. Maraldi, C. Desiderio, F. Vincenzoni, G. La Rocca, F. Alviano, P. Reschiglian, A. Zattoni, B. Roda

Università degli Studi di Bologna

#### **Sala: Convento 5**

#### Sessione Parallela: **Chemiometria e Qualità del Dato (CHEMO)**

Moderatori: Proff. Paolo Oliveri e Davide Ballabio

09.55 – 10.15 **CHEMO-KN01** *CHEMOMETRIC APPROACHES IN METABOLOMICS: AN OVERVIEW DESCRIBING A REAL CASEWORK FOR THE DEVELOPMENT OF AN EFFECTIVE TEST FOR BREAST CANCER SCREENING*

E. Alladio, F. Trapani, L. Castellino, M. Massano, E. Vallariello, M. Vincenti

Università degli Studi di Torino

10.15 – 10.30 **CHEMO-OR01** *THE CLUSTER VALIDITY INDICES: A COMPREHENSIVE REVIEW AND MULTIPLE COMPARISON BY CHEMOMETRICS METHODS*

R. Todeschini, V. Consonni, F. Gosetti, V. Termopoli, D. Ballabio

Università degli Studi di Milano Bicocca

10.30 – 10.45 **CHEMO-OR02** *PROSTATE CANCER DETECTION BY EXCITATION-EMISSION FLUORESCENCE SPECTROSCOPY OF URINE COUPLED WITH CHEMOMETRICS*

E. Mustorgi, C. Durante, C. Malegori, P. Greco, R. Bartoletti, M. Cocchi, M. Casale

Università degli Studi di Genova

#### **10.45 – 11.15 Coffee Break (Patio Convento)**

11.15 – 11.30 **CHEMO-OR03** *NEW STRATEGIES FOR MULTI-BLOCK DATA ANALYSIS BASED ON THE ROSA ALGORITHM*

A. Biancolillo, M. Ryckewaert, S. Mas Garcia, J.M. Roger, F. Marini

Università degli Studi di Roma La Sapienza

11.30 – 11.45 **CHEMO-OR04** *UNTARGETED METABOLOMIC APPROACHES IN FOOD IDENTIFICATION: THE CASE OF PARMIGIANO REGGIANO PDO CHEESE CERTIFICATION "PRODOTTO DI MONTAGNA PROGETTO TERRITORIO"*

C. Durante, P. Becchi, N. Cavallini, C. Citti, F. Savorani, S. Michellini, V. Pizzamiglio, G. Cannazza, M. Cocchi

Università degli Studi di Modena e Reggio Emilia

11.45 – 12.00 **CHEMO-OR05** *DEVELOPMENT OF ULTRASENSITIVE IMMUNOMETRIC BIOELECTRONIC SENSORS WITH EXPERIMENTAL DESIGN*

C. Scandurra, L. Sarcina, P. Bollella, L. Torsi, E. Macchia

Università degli Studi di Bari Aldo Moro

12.00 – 12.15 **CHEMO-OR06** *DESIGN OF EXPERIMENTS AND RESPONSE SURFACE METHODOLOGY AS POWERFUL TOOLS FOR THE OPTIMIZATION OF SAMPLE PREPARATION AND INSTRUMENTAL ANALYSIS*

B. Benedetti, M. Di Carro, E. Magi

Università degli Studi di Genova

12.15 – 12.30 **CHEMO-OR07** *DEVELOPMENT OF A METHOD FOR THE IDENTIFICATION OF DEFECTS IN STEEL COILS BY SEM, IMAGE ANALYSIS AND MULTIVARIATE STATISTICS*

E. Robotti, V. Zippo, M. Barusco, E. Marengo  
Università degli Studi del Piemonte Orientale (Alessandria)

12.30 – 12.45 **CHEMO-OR08** *MULTIVARIATE IMAGE ANALYSIS FOR IN-FIELD QUANTIFICATION OF ANTHOCYANINS CONTENT*

C. Menozzi, R. Calvini, G. Foca, V. Ferrari, M. Calderisi, G. Nigro, P. Tessarin, D. Bossio, A. Ulrici

Università degli Studi di Modena e Reggio Emilia

12.45 – 13.00 **CHEMO-OR09** *FUTURE FOODS: AUTHENTICATION OF CRICKET AND BUFFALO WORM FLOURS FOR HUMAN CONSUMPTION*

A. Biancolillo, M. Foschi, A.A. D'Archivio

Università degli Studi de L'Aquila

13.00 – 14.00 Lunch (libero)

14.00 – 15.00 Sessione POSTER: CHEMO, SEPMS, FORTOX (Sala: Convento 3)

15.00 – 16.00 Assemblee dei Gruppi Divisionali e Interdivisionali

Sala: Duomo

Sala: Convento 2

Sala: Convento 5

15:00 – 15:30 *G.I. Scienza delle Separazioni*

*G.D. Bioanalitica*

*G.D. Spettroscopia Analitica*

15:30 – 16:00 *G.D. Chemiometria*

*G.D. Chimica Analitica Forense*

*G.I. Sensori*

16.15 Gita Sociale (Area Marina Protetta di Milazzo)

19.30 Cena Tipica (Ristorante "Il Paradiso", Capo Milazzo)

## Mercoledì 14 Settembre 2022

Sala: Duomo

09.00 – 09.45 Conferenza Plenaria PL3 – Moderatore: Prof. Luigi Mondello

Prof. Gérard Hopfgartner (University of Geneva, Switzerland)

*SEPARATIONS SCIENCES COUPLED TO MASS SPECTROMETRY FOR THE ANALYSIS OF COMPLEX SAMPLES: CHALLENGES AND OPPORTUNITIES*

**Sala: Duomo**

Sessione Parallela: **Scienza delle Separazioni + Spettrometria di Massa 3 (SEPMS\_3)**

Moderatori: Dott. Danilo Corradini e Prof. Manuel Sergi

09.55 – 10.15 **SEPMS-KN03** *A NEW SYSTEM BASED ON REAL-TIME ELECTRON IONIZATION MASS SPECTROMETRY (REI-MS): DEVELOPMENT AND APPLICATIONS*

A. Arigò, G. Famigliani, P. Palma, A. Cappiello

- 10.15 – 10.30 **SEPMS-OR16** *ADVANCED AMBIENT MASS SPECTROMETRY METHODS: PRINCIPLES AND APPLICATIONS ON FOOD MATRICES*  
D. Mangraviti, F. Rigano, C. Cafarella, P. Dugo, L. Mondello  
Università degli Studi di Messina
- 10.30 – 10.45 **SEPMS-OR17** *PROCESS INTENSIFICATION IN THE BIOPHARMA INDUSTRY: IMPROVING PURIFICATION OF THERAPEUTIC PEPTIDES THROUGH MULTICOLUMN COUNTERCURRENT PREPARATIVE LIQUID CHROMATOGRAPHY*  
M. Catani, C. De Luca, D. Bozza, C. Nosengo, S. Felletti, G. Lievore, T. Müller-Späth, A. Ricci, M. Macis, A. Cavazzini  
Università degli Studi di Ferrara
- 10.45 – 11.15 Coffee Break (Sala: Duomo)**
- 11.15 – 11.30 **SEPMS-OR18** *TRACKING HEME-PROTEIN INTERACTIONS IN HEALTHY AND PATHOLOGICAL HUMAN SERUM IN NATIVE CONDITIONS BY HOLLOW-FIBER FLOW FIELD-FLOW FRACTIONATION WITH MULTIDETECTION*  
V. Marassi, S. Giordani, B. Roda, P. Reschiglian, A. Zattoni  
Università degli Studi di Bologna
- 11.30 – 11.45 **SEPMS-OR19** *CHARACTERIZATION OF ANACARDIC ACIDS IN SAMPLE EXTRACTS OF PISTACHIO (PISTACIA VERA L.) SHELLS BY LIQUID CHROMATOGRAPHY AND TANDEM MASS SPECTROMETRY*  
G. Ventura, F. Rizzo, D. Blasi, D. Mesto, C.D. Calvano, G. Farinola, T.R.I. Cataldi  
Università degli Studi di Bari Aldo Moro
- 11.45 – 12.00 **SEPMS-OR20** *MATRIX SOLID PHASE DISPERSION MICRO EXTRACTION USING DEEP EUTECTIC SOLVENT (DES-MSPD) FOR DETERMINATION OF PESTICIDES IN TOMATOES*  
S. Della Posta, V. Gallo, N. Felli, M. Gherardi, A. Gentili, L. De Gara, C. Fanali  
Università Campus Biomedico di Roma
- 12.00 – 12.15 **SEPMS-OR21** *ADVANCING MOSH/MOAH ANALYSIS TOWARDS SPECIATION AND CONTAMINANTS IDENTIFICATION*  
D. Peroni, A. Carretta  
SRA Instruments
- 12.15 – 12.30 **SEPMS-OR22** *EVALUATION OF VITAMINE D LEVEL IN BIOLOGICAL FLUIDS BY USING GAS CHROMATOGRAPHY TECHNIQUE COUPLED TO TRIPLE QUADRUPOLE MASS SPECTROMETRY*  
G. Micalizzi, D. Donnarumma, C. Buzzanca, F. Vento, L. Mondello  
Università degli Studi di Messina
- 12.30 – 12.45 **SEPMS-OR23** *FFF-BASED HIGH-THROUGHPUT SEQUENCE SHORTLISTING TO SUPPORT THE DEVELOPMENT OF APTAMER-BASED ANALYTICAL STRATEGIES*  
V. Marassi, M. Mattarozzi, L. Toma, S. Giordani, L. Ronda, B. Roda, A. Zattoni, M. Careri, P. Reschiglian  
Università degli Studi di Bologna
- 12.45 – 13.00 **SEPMS-OR24** *A DATA DEPENDENT ACQUISITION-BASED APPROACH FOR THE IDENTIFICATION OF UNKNOWN FAST-ACTING TOXINS AND THEIR METABOLITES IN SHELLFISH*  
L. Tartaglione, F. Varriale, S. Dall'Ara, A. Calfapietra, C. Dell'Aversano

Università degli Studi di Napoli Federico II

13.00 – 13.15 **SEPMS-OR25** *THREE- AND FOUR-DIMENSIONAL GAS CHROMATOGRAPHY-MASS SPECTROMETRY BASED METHODS: WHEN AND WHY TO EXPLOIT*

P.Q. Tranchida, M. Zoccali, M. Galletta, A. Ferracane, A. Arena, L. Mondello

Università degli Studi di Messina

## Sala: Convento 2

Sessione Parallela: **Ambiente e Beni Culturali 3 (AMB\_3)**

Moderatori: Proff. Carlo Dossi e Raffaella Biesuz

09.55 – 10.15 **AMB-KN03** *THE MONITORING OF MICROPLASTICS IN COMPLEX AQUATIC MATRICES: THE POTENTIAL OF NEAR INFRARED HYPERSPECTRAL IMAGING (NIR-HSI) COMBINED WITH CHEMOMETRIC STRATEGIES*

G. Sciutto, S. Piarulli, C. Malegori, F. Grasselli, L. Airoidi, S. Prati, R. Mazzeo, P. Oliveri

Università degli Studi di Bologna

10.15 – 10.30 **AMB-OR16** *PHOTO-SENSITIZING PROPERTIES OF SUWANNEE RIVER NOM AND THEIR EVOLUTION UPON UVC LIGHT*

D. Palma, A. Bianco Prevot, R. Salucci, C. Richard

Università degli Studi di Torino

10.30 – 10.45 **AMB-OR17** *UNTARGETED ANALYSIS OF ENVIRONMENTAL CONTAMINANTS IN SURFACE SNOW SAMPLES OF SVALBARD ISLANDS*

C. Cavaliere, B. Giannelli Moneta

Università degli Studi di Roma La Sapienza

### 10.45 – 11.15 Coffee Break (Patio Convento)

11.15 – 11.30 **AMB-OR18** *CRITICAL EVALUATION OF CHALLENGES AND LIMITATIONS IN POLYURETHANE MICROPLASTICS ANALYSIS BY PY-GC/MS*

I. Coralli, I. Goßmann, D. Fabbri, B.M. Scholz-Böttcher

Università degli Studi di Bologna

11.30 – 11.45 **AMB-OR19** *DETECTION OF SIDEROPHORE-TYPE LIGANDS IN SEA WATER SAMPLES VIA SOLID PHASE EXTRACTION AND HPLC-ESI-MS/MS*

D. Vivado, A. Salis, G. Damonte, E. Millo, A. Parodi, P. Rivaro

Università degli Studi di Genova

11.45 – 12.00 **AMB-OR20** *AN INTEGRATED ANALYTICAL APPROACH TO INVESTIGATE THE INTERACTION BETWEEN MICROPLASTICS AND (TRACE) ELEMENTS IN ENVIRONMENTAL SAMPLES*

G. Binda, S. Carnati, A. Pozzi, D. Spanu, D. Monticelli, C. Dossi, L. Nizzetto

Norwegian Institute for Water Research (NIVA)

12.00 – 12.15 **AMB-OR21** *CONVERSION OF RICE HUSK BIOMASS INTO SORPTIVE CARBON NANOMATERIALS FOR STEROID HORMONES IN ENVIRONMENTAL WATERS*

P. Bianchini, F. Merlo, F. Maraschi, A. Profumo, A. Speltini

Università degli Studi di Pavia

12.15 – 12.30 **AMB-OR22** *MICROPLASTICS: POTENTIAL IMPACTS ON THE OCEAN'S BIOGEOCHEMISTRY*

L. Galgani, M. Consumi, S.A. Loiselle

Università degli Studi di Siena

12.30 – 12.45 **AMB-OR23** *SAMPLING AND MOLECULAR DETECTION OF BIOAEROSOLS: AIRBORNE BACTERIA IN A WASTE WATER TREATMENT PLANT AND SARS-COV-2 IN A POORLY AERATED ROOM WITH A MILD SYMPTOMATIC SUBJECT*

A.S. Gaetano, S. Semeraro, S. Licen, E. Greco, F. Fontana, L. Clemente, L. Zupin, A. Cain, F. Malfatti, A. Pallavicini, M.G. Perrone, A. Miani, P. Piscitelli, P. Barbieri

Università degli Studi di Trieste

12.45 – 13.00 **AMB-OR24** *NON-TARGET SCREENING WITH HIGH RESOLUTION MASS SPECTROMETRY FOR THE IDENTIFICATION OF EMERGING CONTAMINANTS IN SURFACE WATERS AND WASTEWATER EFFLUENTS*

M.H. Belay, D. Papagiannaki, N.P.F. Gonçalves, M. Manfredi, A. Assoumani, R. Binetti, E. Marengo, P. Calza, E. Robotti

Università degli Studi del Piemonte Orientale (Alessandria)

13.00 – 13.15 **AMB-OR25** *VERSATILE FLUORESCENT PIGMENTS FROM AN ANTARCTIC BACTERIUM: CHARACTERIZATION AND ENVIRONMENTAL APPLICATION*

M. Zannotti, S. Pucciarelli, A. Vassallo, R. Giovannetti

Università degli Studi di Camerino

#### **Sala: Convento 5**

##### Sessione Parallela: **Sensori e Biosensori 1 (SENS\_1)**

Moderatori: Proff. Giovanna Marrazza e Marco Giannetto

09.55 – 10.15 **SENS-KN01** *NOT ONLY ELECTROANALYSIS: APPLICATIONS OF PRUSSIAN BLUE DEVELOPING COLORIMETRIC (BIO)SENSORS*

S. Salatiello, A. Zanfardino, M. Spinelli, A. Amoresano, F. Marini, S. Cinti

Università degli Studi di Napoli Federico II

10.15 – 10.30 **SENS-OR01** *BIO-SMART LABELS AND CHEMOMETRICS: MAKING THE DIFFERENCE TOWARDS SUSTAINABLE FRESHNESS MONITORING*

L.R. Magnaghi, C. Zanoni, G. Alberti, P. Quadrelli, R. Biesuz

Università degli Studi di Pavia

10.30 – 10.45 **SENS-OR02** *IMPACT OF THE ANTIGEN SATURATION HOOK EFFECT ON THE DEVELOPMENT OF FIVE LATERAL FLOW IMMUNOASSAYS DETECTING INFECTIOUS DISEASES*

S. Cavallera, F. Di Nardo, T. Serra, V. Testa, M. Chiarello, C. Baggiani, Sergio Rosati, Giulia Pezzoni, Santina Grazioli, and L. Anfossi

Università degli Studi di Torino

#### **10.45 – 11.15 Coffee Break (Patio Convento)**

11.15 – 11.30 **SENS-OR03** *SELF-POWERED ENZYMATIC AND GATE MACHINERY FOR ULTRASENSITIVE DETECTION OF HEPATITIS B VIRUS (HBV)*

A. Imbriano, A. Tricase, E. Macchia, L. Torsi, P. Bollella

Università degli Studi di Bari Aldo Moro

11.30 – 11.45 **SENS-OR04** *ACTIVE PRINCIPLES MONITORING IN PARKINSON'S DRUGS VIA EASY, LOW-COST AND FAST-RESPONSE COLORIMETRIC DETECTION STRATEGIES*

M. Lettieri, P. Palladino, S. Scarano, M. Minunni

Università degli Studi di Firenze



- 11.45 – 12.00 **SENS-OR05** PROGRAMMABLE CELL-FREE TRANSCRIPTIONAL SWITCHES FOR ANTIBODIES DETECTION  
S. Bracaglia, A. Patino Diaz, S. Ranallo, T. Patino, A. Porchetta, F. Ricci  
 Università degli Studi di Roma Tor Vergata
- 12.00 – 12.15 **SENS-OR06** LASER PATTERNING FOR CONTROLLED METAL NANOSTRUCTURES FORMATION FOR SENSORS DEVELOPMENT  
A. Scroccarello, F. Della Pelle, F. Silveri, D. Compagnone  
 Università degli Studi di Teramo
- 12.15 – 12.30 **SENS-OR07** A 3K-TOOLBOX TO TRIGGER EPITOPE IMPRINTING INTO BIOPOLYMERS: THE CASE STUDY OF PD-L1 SENSING DETECTION  
F. Torrini, P. Palladino, G. Goletta, M. Minunni, S. Scarano  
 Università degli Studi di Firenze
- 12.30 – 12.45 **SENS-OR08** GOLD SURFACES FOR BIOSENSOR APPLICATION: BIO-FUNCTIONALIZATION AND ANALYTICAL DETECTION OF VIRAL NUCLEOCAPSID PROTEINS  
L. Sarcina, F. Torricelli, E. Macchia, L. Torsi  
 Università degli Studi di Bari Aldo Moro
- 12.45 – 13.00 **SENS-OR09** GRAPHENE-PAPER ELECTRODES AS NEW PLATFORMS FOR AMPEROMETRIC BIOSENSING  
L. Malavolta, F. Poletti, A. Scidà, L. Lancellotti, B. Zanfrognini, F. Valorosi, A. Kovtun, V. Palermo, E. Treossi, C. Zanardi  
 CNR Bologna
- 13.00 – 13.15 **SENS-OR10** PLASMONIC APTASENSOR WITH FUNCTIONAL ANTIFOULING SURFACE FOR DETECTION OF LYSOZYME IN REAL MATRIX  
N. Bellassai, R. D'Agata, G. Spoto  
 Università degli Studi di Catania
- 13.15 – 14.00 Lunch (libero)
- 14.00 – 15.00 SESSIONE POSTER: SENS, SENSEL, ALI, GREEN (Sala: Convento 3)

**Sala: Duomo**

Sessione Parallela: **Sensori e Biosensori 2 (SENS\_2)**

Moderatori: Proff. Danila Moscone e Maria Minunni

- 15.00 – 15.20 SENS-KN02 PAPER-BASED ELECTROCHEMICAL (BIO)SENSORS AS SMART AND SUSTAINABLE DEVICES  
 L. Fabiani, D. Moscone, S. Fillo, R. De Santis, F. Lista, F. Arduini  
 Università degli Studi di Roma Tor Vergata
- 15.20 – 15.35 SENS-OR11 IMPLEMENTATION OF EXPERIMENTAL DESIGN TECHNIQUES TO OPTIMIZE IMMUNOGLOBULINS DETECTION WITH SIMOA SP-X SYSTEM  
C. Scandurra, P. Bollella, L. Torsi, E. Macchia  
 Università degli Studi di Bari Aldo Moro
- 15.35 – 15.50 SENS-OR12 NEW APPROACHES FOR ELECTROCHEMICAL DEVICES DEVELOPMENT BASED ON CARBONACEOUS MATERIALS INTEGRATING GOLD NANOSTRUCTURES  
F. Silveri, F. Della Pelle, A. Scroccarello, M. Del Carlo, D. Compagnone

Università degli Studi di Teramo

15.50 – 16.05 SENS-OR13 *JAGGED1-FLUC: A BIOLUMINESCENT RECOMBINANT PROTEIN AS A NEW TOOL TO IMPROVE THE EARLY DETECTION AND DIAGNOSIS OF COLORECTAL CANCER*

A. Punzo, A. Silla, R. Comito, P. Simoni, A. Roda, C. Caliceti

Università degli Studi di Bologna

16.05 – 16.20 SENS-OR14 *POINT-OF-CARE DETECTION OF SARS-COV-2: A NOVEL IMMUNOSENSOR USABLE AT VOLTAMETRIC AND IMPEDIMETRIC TRANSDUCTION MODE*

C. Tortolini, A.E.G. Cass, R. Antiochia

Università degli Studi di Roma La Sapienza

16.20 – 16.35 SENS-OR15 *HYBRID Au@SiNWs ARRAYS AS POTENTIAL SERS PLATFORMS: SYNTHESIS, ANALYTICAL CHARACTERIZATION AND APPLICATION*

R.A. Picca, M. Izzì, A.A. Leonardi, M.C. Sportelli, M.J. Lo Faro, B. Fazio, A. Irrera, N. Cioffi

Università degli Studi di Bari Aldo Moro

16.35 – 17.00 Coffee Break (Sala: Duomo)

17.00 – 17.15 SENS-OR16 *SMART AND PORTABLE ELECTROCHEMICAL IMMUNOSENSOR FOR SARS-COV-2 SPIKE PROTEIN DETECTION WITH MACHINE LEARNING FEATURES*

S. Fortunati, C. Giliberti, M. Giannetto, A. Bolchi, D. Ferrari, G. Donofrio, V. Bianchi, A. Boni, I. De Munari, M. Careri

Università degli Studi di Parma

17.15 – 17.30 SENS-OR17 *ELECTROCHEMICAL LATERAL FLOW IMMUNOASSAY FOR IMPROVED ANALYTICAL PERFORMANCES*

F. Di Nardo, A. Miglione, S. Cavallera, T. Serra, C. Baggiani, S. Cinti, L. Anfossi

Università degli Studi di Torino

17.30 – 17.45 SENS-OR18 *DISPOSABLE LABEL-FREE VOLTAMMETRIC IMMUNOSENSOR FOR SENSITIVE DETECTION OF INTERLEUKIN-6*

L. Micheli, R. Cancelliere, G. Contini, E. Signori

Università degli Studi di Roma Tor Vergata

17.45 – 18.00 SENS-OR19 *A NOVEL INTEGRATION OF MOLECULARLY IMPRINTED POLYMERS WITH NANOPOROUS SILICON FOR SELECTIVE AND SENSITIVE OPTICAL DETECTION OF PROTEINS*

E. Mazzotta, T. Di Giulio, M. Corsi, S. Mariani, C. Malitesta, G. Barillaro

Università degli Studi del Salento (Lecce)

## **Sala: Convento 2**

Sessione Parallela: **Alimenti e Nutraceutici (ALI)**

Moderatori: Proff. Paola Dugo e Chiara Cordero

15.00 – 15.20 ALI-KN01 *UNLOCKING THE FUTURE OF COMPREHENSIVE TWO-DIMENSIONAL CHROMATOGRAPHY IN FOOD-OMICS BY ARTIFICIAL INTELLIGENCE ALGORITHMS*

C. Cordero, S. Squara, A. Caratti, E. Alladio, M. Vincenti, Humberto Bizzo, Stephen E. Reichenbach, C. Bicchi

Università degli Studi di Torino

15.20 – 15.35 ALI-OR01 *IDENTIFICATION OF MARKER PEPTIDES OF SPIRULINA ALLERGENS BY LC-ESI-MS/MS*

M. Bianco, G. Ventura, C. D. Calvano, I. Losito, T.R.I. Cataldi

Università degli Studi di Bari Aldo Moro

- 15.35 – 15.50 ALI-OR02 *ANALYTICAL COMPOSITION OF FLOURS THROUGH THERMOGRAVIMETRIC AND RHEOLOGICAL COMBINED METHODS*  
M. Consumi, G. Leone, S.A. Loïselle, A. Magnani, G. Tamasi  
 Università degli Studi di Siena
- 15.50 – 16.05 ALI-OR03 *ECOLOGICAL TRANSITION IN THE FIELD OF PACKAGING AND FOOD CONTACT MATERIALS: NEW TRENDS AND EVALUATION OF SAFETY AND STABILITY*  
A. Cavazza, M. Grimaldi, O. Pitirollo, E. Messinese, C. Sciancalepore, D. Milanese  
 Università degli Studi di Parma
- 16.05 – 16.20 ALI-OR04 *POMEGRANATE SEEDS: A HIGH INTEREST FOOD WASTE*  
 F. Cairone, C. Salvitti, A. Iazzetti, G. Fabrizi, F. Pepi, S. Cesa  
 Università degli Studi di Roma La Sapienza
- 16.20 – 16.35 ALI-OR05 *THE COUPLING OF ENANTIO-SELECTIVE GAS CHROMATOGRAPHY TO ISOTOPIC RATIO MASS SPECTROMETRY: PERSPECTIVES AND THE NEED OF MULTIDIMENSIONAL GAS-CHROMATOGRAPHIC APPROACHES*  
 L. Cucinotta, G. De Grazia, D. Sciarrone, L. Mondello  
 Università degli Studi di Messina
- 16.35 – 17.00 Coffee Break (Patio Convento)
- 17.00 – 17.15 ALI-OR06 *RELEASE OF SELECTED NON-INTENTIONALLY ADDED SUBSTANCES (NIAS) FROM PET FOOD CONTACT MATERIALS: A NEW ONLINE SPE-UHPLC-MS/MS MULTIRESIDUE METHOD*  
 R. Aigotti, N. Giannone, A. Asteggiano, E. Mecarelli, C. Medana, F. Dal Bello  
 Università degli Studi di Torino
- 17.15 – 17.30 ALI-OR07 *EXTENDED AUTOMATION OF OLIVE OIL ANALYSIS ACCORDING TO CE REGULATION 2568/91*  
A. Carretta, C. Ventre  
 SRA Instruments
- 17.30 – 17.45 ALI-OR08 *DETAILED ELUCIDATION OF INTACT LIPIDS IN FUNCTIONAL FOODS BY EXPLOITING A NOVEL IDENTIFICATION APPROACH BASED ON LINEAR RETENTION INDEX IN LIQUID CHROMATOGRAPHY – MASS SPECTROMETRY*  
F. Rigano, P. Dugo, L. Mondello  
 Università degli Studi di Messina
- 17.45 – 18.00 ALI-OR09 *COMBINED ANALYTICAL APPROACHES FOR CARBOHYDRATE CHARACTERIZATION OF LOW-FODMAP PRODUCTS AND NUTRACEUTICAL EXTRACTS FROM AGRO-INDUSTRIAL BY-PRODUCTS*  
O. Pitirollo, M. Grimaldi, S. De Luca, G. D'Ambrosio, S. Pironi, A. Cavazza  
 Università degli Studi di Parma

**Sala: Convento 5**

Sessione Parallela: **Forense e Tossicologia (FORTOX)**

Moderatori: Proff. Marco Vincenti e Alessandro Giuffrida

- 15.00 – 15.20 FORTOX-KN01 *THE CHALLENGING SPECIATION OF Cr(VI) IN TOXICOLOGICAL RELEVANT MATRICES: WHERE OFFICIAL METHODS CAN FAIL AND HOW TO DEVELOP ALTERNATIVE PROTOCOLS*  
S. Recchia, D. Spanu, C. Dossi, A. Spinazzè, A. Cattaneo, D.M. Cavallo

- 15.20 – 15.35 FORTOX-OR01 *IDENTIFICATION, CHARACTERIZATION AND PRESUMPTIVE TEST DEVELOPMENT OF A NEW SYNTHETIC CANNABINOID IN FORESIC CASEWORK*  
V. Greco, A. B. Carbonaro, V. Buccilli, P. Maida, A. Giuffrida  
Università degli Studi di Catania
- 15.35 – 15.50 FORTOX-OR02 *MULTICLASS ANALYSIS OF PSYCOACTIVE SUBSTANCES FROM HAIR BY PARALLEL ARTIFICIAL LIQUID MEMBRANE EXTRACTION AND UPLC-MS/MS DETECTION*  
C. Montesano, Y. Dragt, M. Carrella, F. Vincenti, M. Croce, G. Di Francesco, M. Sergi, R. Curini  
Università degli Studi di Roma La Sapienza
- 15.50 – 16.05 FORTOX-OR03 *DETERMINATION OF CHIRAL PESTICIDES IN HEMP SEEDS WITH SUPERCRITICAL FLUID CHROMATOGRAPHY*  
M. Russo, M.R. Testa Camillo, P. Dugo, L. Mondello  
Università degli Studi di Messina
- 16.05 – 16.20 FORTOX-OR04 *DEVELOPMENT OF BIODEGRADABLE SAMPLING DEVICES FOR THE ANALYSIS OF ILLICIT DRUG IN ORAL FLUIDS*  
F. Vincenti, M. Croce, G. Di Francesco, C. Montesano, R. Curini, D. Compagnone, M. Sergi  
Università degli Studi di Roma La Sapienza
- 16.20 – 16.35 FORTOX-OR05 *GC-FTIR AFFORDS RELIABLE FORENSIC EVIDENCE FOR DRUGS OF ABUSE*  
P. Donato, T.M.G. Salerno, C. Coppolino, L. Mondello  
Università degli Studi di Messina
- 16.35 – 17.00 Coffee Break (Patio Convento)
- 17.00 – 17.15 FORTOX-OR06 *EXPOSURE ASSESSMENT OF NITRITE AND NITRATE INTAKE FROM LEAFY VEGETABLES CONSUMPTION THROUGH MARGIN OF SAFETY (MOS) EVALUATION*  
G. Berardi, A. Di Taranto, V. Vita, G. Rizzi, M. Iammarino  
IZSPB Foggia
- 17.15 – 17.30 FORTOX-OR07 *UNVEILING THE OXYGEN HETEROCYCLIC FRACTION OF CITRUS SCENTED HAND GEL SANITIZERS BY MEANS OF HPLC-MS/MS: FOCUS ON COUMARINS AND FUROCOUMARINS CONTENT AND SKIN RISK TO EXPOSURE*  
T.M.G. Salerno, G. Cafeo, P. Dugo, L. Mondello  
Università degli Studi di Messina
- 17.30 – 17.45 FORTOX-OR08 *A MICROFLUIDIC PAPER-BASED DEVICE FOR ESTIMATING THE POST-MORTEM INTERVAL (PMI): AMMONIUM ANALYSIS IN VITREOUS HUMOR*  
G. Musile, Y. Agard, E.F. De Palo, F. Bortolotti, F. Tagliaro  
Università degli Studi di Verona
- 21.00 Cena Sociale (Ristorante "La Baia", Capo Milazzo)

## Giovedì 15 Settembre 2022

Sala: Duomo

- 09.00 – 09.45 Conferenza Plenaria PL4 – Moderatore: Prof. Dario Compagnone  
Prof. Jesús Alberto Escarpa Miguel (University of Alcalá, Spain)  
*MICROMOTORS FOR (BIO)-SENSING AND ENVIRONMENTAL APPLICATIONS: EXPLORING THEIR COLLECTIVE BEHAVIOR*

**Sala: Duomo**

Sessione Parallela: **Sensori ed Elettroanalitica (SENSEL)**

Moderatori: Proff. Domenica Tonelli e Fabiana Arduini

- 09.55 – 10.15 SENSEL-KN01 *PROTEIN RECOGNITIVE SOFTMOLECULARLY IMPRINTED NANOPARTICLES FOR PLASMONIC SENSING PLATFORMS*  
N. Cennamo, L. Zeni, A.M. Bossi  
Università degli Studi di Verona
- 10.15 – 10.30 SENSEL-OR01 *INVESTIGATION OF THE SALT EFFECT AFFECTING THE ACCURACY OF pH COLORIMETRIC SENSOR ARRAYS (CSAS)*  
A. Pastore, D. Badocco, L. Cappellin, P. Pastore  
Università degli Studi di Padova
- 10.30 – 10.45 SENSEL-OR02 *ELECTROCHEMICAL GATING FOR SENSING: OPERATING PRINCIPLE AND WEARABLE DEVICES*  
I. Gualandj, F. Mariani, D. Arcangeli, M. Serafini, M. Tessarolo, F. Decataldo, L. Possanzini, T. Cramer, B. Fraboni, D. Tonelli, E. Scavetta  
Università degli Studi di Bologna
- 10.45 – 11.15 Coffee Break (Sala: Duomo)
- 11.15 – 11.30 SENSEL-OR03 *A PAPER ORIGAMI PLATFORM FOR ELECTROCHEMICAL QUALITY CONTROL OF AGRI-FOOD WASTE*  
N. Colozza, E. Di Meo, D. Moscone, F. Arduini  
Università degli Studi di Roma Tor Vergata
- 11.30 – 11.45 SENSEL-OR04 *SEWAGE SLUDGE-DERIVED BIOCHAR TO DESIGN MORE SUSTAINABLE SENSORS FOR ELECTROANALYTICAL APPLICATIONS*  
P.S. Sfragano, S. Laschi, L. Renai, M. Fichera, M. Del Bubba, I. Palchetti  
Università degli Studi di Firenze
- 11.45 – 12.00 SENSEL-OR05 *DISSIPATIVE DNA NANOTECHNOLOGY FOR ANALYTICAL CHEMISTRY*  
E. Del Grosso, L. J. Prins, F. Ricci  
Università degli Studi di Roma Tor Vergata
- 12.00 – 12.15 SENSEL-OR06 *POTENTIOMETRY UNRAVELS THE REACTION MECHANISM IN SEMICONDUCTOR PHOTOCATALYSIS*  
F. Sordello, C. Minero  
Università degli Studi di Torino
- 12.15 – 12.30 SENSEL-OR07 *A PRINTED MICROFLUIDIC WEARABLE SENSOR FOR CORTISOL MONITORING IN SWEAT*

L. Fiore, V. Mazzaracchio, A. Serani, L. Fabiani, A. Barba, G. M. Bianco, G. Marrocco, D. Moscone, F. Arduini

Università degli Studi di Roma Tor Vergata

12.30 – 12.45 SENSEL-OR08 *LAYERED DOUBLE HYDROXIDES FOR ELECTROANALYTICAL APPLICATIONS*

D. Tonelli, E. Scavetta, I. Gualandi, F. Mariani, D. Arcangeli

Università degli Studi di Bologna

12.45 – 13.00 SENSEL-OR09 *RATIONAL DESIGN OF HAIRPIN DNA REPORTERS FOR ENHANCED CRISPR-BASED DETECTION OF VIRUSES AND BACTERIA*

M. Rossetti, N. Bagheri, F. Ricci, A. Porchetta

Università degli Studi di Roma Tor Vergata

## Sala: Convento 2

Sessione Parallela: **Green Chemistry (GREEN)**

Moderatori: Proff. Alessandra Gentili e Chiara Fanali

09.55 – 10.15 GREEN-KN01 *ADVANCED ANALYTICAL TOOLS BASED ON COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY COUPLED WITH MASS SPECTROMETRY FOR THE ANALYSIS OF BIOMASS AND PLASTIC WASTE SAMPLES*

M. Beccaria, M. Piparo, Y. Zou, P.H. Stefanuto, G. Purcaro, A.L. Mendes Siqueira, A. Maniquet, P. Giusti, J.F. Focant, A. Cavazzini

Università degli Studi di Ferrara

10.15 – 10.30 GREEN-OR01 *ANALYTICAL PYROLYSIS-GAS CHROMATOGRAPHY COUPLED WITH MASS SPECTROMETRY (PY-GC/MS) TO CHARACTERISE LIGNIN EXTRACTED WITH DEEP EUTECTIC SOLVENTS*

M. Mattonai, F. Nardella, G. Messina, E. Ribechini

Università degli Studi di Pisa

10.30 – 10.45 GREEN-OR02 *DETERMINATION OF PESTICIDES IN FOOD SAMPLES THROUGH REDUCED SAMPLE PREPARATION COUPLED TO FLOW- AND CRYOGENIC-MODULATION GC×GC COMBINED WITH TRIPLE-QUADRUPOLE MASS SPECTROMETRY*

M. Zoccali, A. Arena, A. Ferracane, P.Q. Tranchida, L. Mondello

Università degli Studi di Messina

10.45 – 11.15 Coffee Break (Patio Convento)

11.15 – 11.30 GREEN-OR03 *DEVELOPMENT OF AN ADVANCED EXTRUSION PROCESS FOR THE REDUCTION OF VOLATILE AND SEMI-VOLATILE ORGANIC COMPOUNDS OF RECYCLED HDPE FROM FUEL TANKS*

M. Roncoli, M. Monti, E. Perin, E. Conterosito, U. Romagnolli, B. Muscato, M. Giroto, M. T. Scrivani, V. Gianotti

Università degli Studi del Piemonte Orientale (Alessandria)

11.30 – 11.45 GREEN-OR04 *DEVELOPMENT OF A PROTOTYPE SYSTEM BASED ON HIGH TEMPERATURE LIQUID CHROMATOGRAPHY FOR THE DETERMINATION OF PARABENS IN COSMETIC AND FOOD SAMPLES*

R. La Tella, F. Rigano, L. Mondello

Università degli Studi di Palermo

11.45 – 12.00 GREEN-OR05 *A SUSTAINABLE STRATEGY FOR THE PRODUCTION OF BIOETHANOL FROM CITRUS WASTE*

R. Vadalà, G. Lo Vecchio, A. Macrì, L. Messina, M. Porretti, R. Rando, N. Cicero, G. Dugo, R. Costa

Università degli Studi di Messina

12.00 – 12.15 GREEN-OR06 *COMPARISON OF RETENTION BEHAVIOR OF NATURAL CANNABINOIDS IN SUPERCRITICAL FLUID CHROMATOGRAPHY AND NORMAL PHASE LIQUID CHROMATOGRAPHY*

A. Buratti, S. Felletti, M. Catani, C. De Luca, G. Compagnin, F. Gasparrini, G. Mazzocanti, A. Cavazzini

Università degli Studi di Ferrara

12.15 – 12.30 GREEN-OR07 *GREEN EXTRACTIONS OF AROMATIC HERBS AND CITRUS WASTE: NOVEL CONFIGURATIONS FROM LAB TO PILOT PLANT SCALE*

J. Gonzalez-Rivera, B. Campanella, E. Pulidori, L. Bernazzani, M. Onor, P. Bàrberi, M. R. Tiné, C. Duce, E. Bramanti, C. Ferrari

CNR Pisa

12.30 – 12.45 GREEN-OR08 *THE EUTECTIC MIXTURE FORMED BY L-MENTHOL AND BUTYLATED HYDROXYTOLUENE: A GREEN ANTIOXIDANT SOLVENT FOR THE SUSTAINABLE EXTRACTION OF FAT-SOLUBLE MICRONUTRIENTS FROM RAW AND COOKED FOODSTUFF*

A. Gentili, C. Dal Bosco

Università degli Studi di Roma La Sapienza

12.45 – 13.00 GREEN-OR09 *QUANTIFICATION OF VOLATILE MARKER COMPOUNDS IN FLAVORED CITRUS PRODUCTS BY USING SPME-GC/MS*

E. Trovato, F. Vento, P. Dugo, L. Mondello

Università degli Studi di Messina

Sala: Duomo

13.00 – 13.15 Chiusura del Congresso





Società Chimica Italiana  
Divisione di Chimica  
Analitica

**XXIX Congresso della Divisione di Chimica Analitica**

## **Plenary Lectures**

## **MICROPLASTICS IN THE AQUATIC ENVIRONMENT: GREEN ANALYTICAL PROTOCOLS, RISKS AND SUSTAINABLE SOLUTIONS**

*Damià Barceló<sup>1,2</sup>*

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Plastic pollution is nowadays a global and ubiquitous problem being detected everywhere: marine environment, sand beaches, wastewaters, surface waters, soils, sludges, sediments, biota, food and air. The word plastic comes from the Greek term *plastikos*, which means that it can remain shaped in various systems. Global plastic production did hit approximately 348 million tonnes in 2017, being China the largest producer responsible of 27% of worldwide pollution. It is estimated that more than 8300 million tonnes of virgin plastic have been produced to date. Many consumers are not aware that plastic goods are usually made in petrochemical plants. According to the 2019 Centre for International Environmental Law Report, its production will contribute approximately to 850 million tons greenhouse emissions. Plastic is part of our daily life and worldwide we use 4 trillion plastic bags annually and 1 million plastic bottles every minute .

Plastics in the environment are divided into Macro-Plastics (with particles >2.5 cm), Meso-Plastic ( with particles 2.5cm-5mm) , Micro- Plastics (MPs) (with particles between micron- 5mm) and Nano-Plastics ( with particles between 1-100nm). Macro-Plastics include everything identified as litter, such as plastic bags, bottles discarded fishing nets, plastic toys among other items and they can be usually observed. MPs are commonly invisible to the naked eye. particularly when mixed with sediment. Macro-Plastics enter the marine environment via rivers, poor waste management or being dumped into the marine waters.

MPs are directly released into the water or formed by degradation of Macro-plastics. In short, annually between 4 and 12 millions of tonnes of plastics are going into the oceans and most probably in 2050 will exceed the amount of fish. The amount of anthropogenic debris in the marine and coastal environments is steadily increasing with an estimation of 270,000 of plastic floating. In consequence international organizations, as well as NGOs recognize marine litter as a global issue of major concern. Plastic litter enters the marine environment from diverse points and diffuse sources and it can be

transported through rivers long distances before being deposited in the bottom of seas. Few studies suggest that river litter can contribute up to 40% of all marine litter input, being estimated over 1.2-2.5 million tonnes of plastic every year (1,2).

MPs are made from diverse molecules and correspond to diverse product types. MPs are composed of diverse suite of polymer type, being the most produced and consumed ones polypropylene (PP), low density polyethylene (LDPE), high density polyethylene (HDPE), polyvinyl chloride (PVC), polyurethane, polyethylene terephthalate (PET), polystyrene (PS) and polyamide (PA) are diverse and come from a multitude of sources, also they are in different sizes, colours, shapes and types of materials. MPs contain additives, i.e. phthalates and they can be as well a vector of organic contaminants and pathogens that can be ingested by organisms and introduced into the food web. Airborne fibrous MPs may enter our respiratory system with risk to the environment and humans.

Having said that, this presentation will cover in the first part different aspects of MPs and Macro-Plastic litter pollution in coastal waters, rivers, sediments and lakes. Case studies of MP pollution in several coastal environments, sediments and catchments of China, Saudi Arabia, India, Europe and Australia will be reported (2,3,4). It is well-known that MPs affect communities, biological diversity, and ecosystem processes will be reported. MPs increase the abundance of some taxa but decrease the abundance of some other taxa, indicating trade-offs among taxa and altered microbial community composition in both the natural environment and animals' gut. The alteration of community composition by microplastics is highly conserved across taxonomic ranks, while the alpha diversity of microbiota is often reduced or increased, depending on the microplastics dose and environmental conditions, suggesting potential threats to biodiversity. Biogeochemical cycles, greenhouse gas fluxes, and atmospheric chemistry, can also be altered by microplastics pollution. These findings suggest that microplastics may impact the U.N. Sustainability Development Goals (SDGs) to improve atmospheric, soil, and water quality and sustaining biodiversity (5).

The second part of this lecture will discuss green analytical chemistry (GAC) protocols for the analysis of MPs in water (6,7). Within the last years aspects such as green, eco-friendly and sustainable are making their way into analytical chemistry. The field has changed with the introduction of these concepts. Information on the consumption of toxic solvents and energy is now a part of everyday life. This green analytical chemistry could be playing a pioneering role in the analysis of micro(nano)plastics, MP(NPs) in the environment. We discuss the roles of green analytical and sustainability within MP(NPs) determination and its possible applications. We explain its many advantages, like their function to preserve the environment and operator health or their role in the so called eco-friendly methodologies, but we also highlight points such as an efficiency in the determination that should be

viewed critically. Finally, we describe how micro(nano)plastics analysis is implementing the GAC and the challenges faced. We would like to emphasize here the importance of implementing GAC for determination micro(nano)plastics to ensure that the intensive study of these contaminants carried out nowadays does not become a source of other pollutants that can be even worse. These aspects have hardly been considered in the methods developed so far and they should. It is important to know that in all steps of the method (i) sampling, (ii) sample preparation and (iii) identification and quantification measures can be taken to make the method more environmentally friendly and sustainable, safer for the operator. In this sense the Green Analytical Chemistry (GAC) aspects have been little considered in the MPs analysis. In the end, GAC has to be considered as a more global aspect, in which

laboratory safety, good practices, and the implementation of systems for the proper disposal of waste and equipment can play a more important role than the reagents used in a particular analysis. The impact that the methodologies used to isolate and determine MPs and NPs have on the environment due to the consumption of reagents and energy and the generation of waste will be addressed as well in this presentation. Examples reported will include analytical methodologies applied to MPs and NPs determination assessing their greenness through National Environmental Methods Index, Analytical Eco-scale and AGREE metrics and discussing green issues related to the consumption of solvents/reagents, etc. In the greenness of analytical methods for micro(nano)plastics several issues that can contribute to that remain to be addressed such as in situ sampling, use of direct methods, miniaturization and automation of methods, replacement of hazardous reagents by less hazardous ones, and application of chemometrics to reduce the number of samples analyzed. It is to be hoped that future studies dedicated to the analysis of these compounds will address this problem and implement these solutions for a more sustainable future.

The last part of this lecture will discuss as well plastic litter and its increase use under Covid-19 outbreak. In this sense the excessive use and consumption of single-use plastics (including personal protective equipment such as masks and gloves) due to COVID-19 pandemic. This review aimed to provide an integrative and synthesized overview on the effects of COVID-19 on macroplastic pollution and its potential implications on the environment and human health in a long-term scenario; addressing the main challenges and discussing potential strategies to potentially overcome them. It emphasizes that future measures, involved in emergent health crisis or not, should reflect the balance between public health and environmental safety as they are both undoubtedly connected. Although the use and consumption of plastics significantly improved our quality of life, it is crucial to shift towards sustainable alternatives, such as bio-based plastics. Plastics should remain in the top of the political agenda in Europe and across the world, not only to

minimize plastic leakage and pollution, but to promote sustainable growth and to stimulate both green and blue- economies (8).

Landfilling and illegal waste disposal have risen to deal with the Covid-19 potentially infectious waste, particularly in developing countries. The intense use of such a disposal method drives us apart from the envisioned 2030 circular economy and environmental sustainability. It is estimated that 3.5 million metric tons of masks have been landfilled worldwide in the first year, which can generate up to  $2.3 \times 10^{21}$  microplastics of  $7 \mu\text{m}$  in the coming year. This presentation addresses the challenges raised in the pandemic and post-pandemic scenarios on landfills; while discussing the potential environmental and health implications that might drive us apart from the 2030 U.N. sustainable goals. Also, it highlights some innovative mitigation technologies and improved management strategies that can pave the way to environmental recovery (9).

MPs and macro litter pollution is nowadays in the radar not only of the scientific community but also of the public, the so-called citizen science. Media coverage helps to push such initiatives being complementary to scientific approaches. Such synergistic combination of academia, the public as well as policy actions should help to mitigate MP and macroplastics litter pollution in the next coming years. It

is important to note that there are still many gaps in knowledge and we do not know well how plastics and MPs are transported and distributed and in what quantity. However, all these programs together with the modelling will allow us to know it soon. Monitoring and sampling systems also need to be improved especially in coastal areas and all existing programs will help. This is urgently needed in the next coming years due to the impact of plastic waste due to Covid-19 outbreak. Previous monitoring program will need to be repeated again to measure and evaluate in the field the real impact of plastic under almost two years of pandemic.

The detailed study of the strictly technological alternatives to the solution of this problem, show that they are not sufficient, and that in many cases they just transfer the problem of water to the generated sludge. More studies in these aspects are absolutely necessary, because although the number of publications is enormous, the gaps in knowledge are also enormous. Another solution that is expected to be developed and that could bring more definitive results is the degradation of MPs by microorganisms (fungi and bacteria). Many studies are being carried out, although the complexity of these studies means that progress in this field is slow. In this context, we would like to add few recommendations : (i) law and waste management strategies, such as exploring new removal technologies and avoid landfilling if this is economically feasible (ii) education, outreach and awareness, (iii) source identification, (iv) increasing monitoring and risk assessment to better understand the threat to biodiversity by reporting additional case studies where showing the impact of MP around the globe and (v) further innovative

research lines like the development of bioplastics to replace SUPs in our daily life. In short a greater awareness and responsibly of the general public, stakeholders, industries is needed. Discussions on this topic, particularly considering the excessive use of plastic, should start soon with the involvement of the scientific community, plastic producers and politicians in order to be prepared for the near future.

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## **APTAMERS – NEW ASPECTS OF THEIR USE IN ELECTRONIC SENSING**

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DNA-based aptamers have become interesting bioreceptors in diagnostic platforms, with excellent selectivity for their target molecules, able to ensure sensitive and specific analysis in biological fluids such as blood, serum, saliva, sweat, or urine. Very attractive was the observation that aptamers can be raised by SELEX also against small analytes, a notorious challenge for other receptor systems and sensor formats. On the other hand, a general concern were the negative charges of the sugar- phosphate backbone of DNA-aptamers that might impact their performance as receptors for biosensing applications in clinical settings as their binding properties might depend on details of the ionic milieu, e.g., the pH and the ionic strength, of the analyte solution.

Hence, the question came up whether significant benefits for the operation of aptamer-based sensors in analyte cocktails of varying ionic properties might come from the use of DNA mimics such as peptide nucleic acids (PNAs) as aptamer receptor systems. But what would happen to the sensing mechanism in electronic sensing, where the potential reorganization of the aptamer upon binding its ligand is believed to lead to a strongly enhanced reorganization of the interfacial charge distribution resulting in the sensitive detection of bio-affinity reactions in these types of sensors.

These questions will be addressed in this talk, and new perspectives offered by PNA-bioreceptors coupled to graphene-based field effect transistors (gFETs) as transducers will be discussed with specific emphasis on the sensing of thrombin and of cardiac troponin I (cTnI, cf. Fig. 1) as clinically relevant analytes.

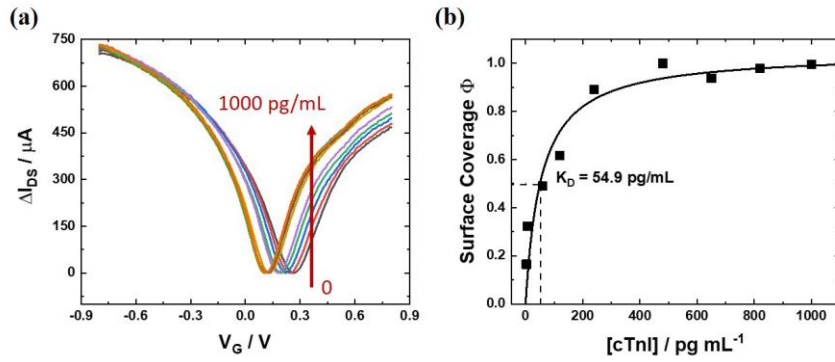


Figure 1: An example for the electronic detection of cTnI in buffer solutions, varying in concentration from 0 to 1000 pg/mL. (a) Series of  $I_{DS}V_G$  curves at different cTnI concentrations; (b) Langmuir isotherm analysis of the data from (a).



**SEPARATIONS SCIENCES COUPLED TO MASS SPECTROMETRY  
FOR THE ANALYSIS OF COMPLEX SAMPLES: CHALLENGES  
AND OPPORTUNITIES***Gérard Hopfgartner*

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With the introduction in the late nineties of atmospheric pressure ionisation, and in particular electrospray (ESI), liquid chromatography coupled to tandem mass spectrometry (LC-MS) has become a key analytical technique where molecular ions are selected and fragmented, often by collisional induced dissociation (CID). This is the basis of highly specific assays that are widely used in drug discovery and development, toxicology, doping control, etc. and enables applications such as proteomics and metabolomics. However, comprehensive qualitative and quantitative analysis of complex biological samples suffers from several constraints such as: limited sample throughput, large number of analytes, large chemical space, isomeric and isobaric analytes, lack of standards and high concentration dynamic range of analytes calling for advanced and integrated analytical workflows. High resolution mass spectrometry and in particular data independent acquisition (DIA) has become a straightforward way to monitor analytes expression in complex systems using various chromatographic setup including RPLC, HILIC and supercritical fluids chromatography. Due to the nature of ESI, annotating of mass spectra becomes also essential for features reduction but remains a challenging problem for quantitative analysis due to the multiple processes occurring during ionization. While ESI is largely applied for the detection of functionalized compounds the use of atmospheric pressure photoionization (APPI) has mainly been described with dopants for the analysis of compounds lacking of functional groups. With APPI depending on the ionization conditions either radical cation or protonated ions can be formed selectively for a large polarity range. Radical cation precursors can further be fragmented by collision induced dissociation and the spectra showed numerous fragments common to that observed in EI spectra enlarging the use of MS libraries.

### PL3

The integration of ion mobility spectrometry, based on the shape of the charged analytes, with mass spectrometry (IMS-MS) enables an additional separation dimension, in particular for isobaric and isomeric analytes. Open port probe (OPP) offers a better alternative to flow injection analysis (FIA) for the development of fast assays also in combination with IMS..

Beside workflows, ionisation control and data analysis optimization instrumental development are essential to obtain more molecular information in a single LC-MS analysis. Furthermore, electron induced dissociation or electron capture dissociation (ExD) and photodissociation (UVPD) extend the possibility for sensitive analyte identification and quantification, combined with IMS.

## MICROMOTORS FOR (BIO)SENSING AND ENVIRONMENTAL APPLICATIONS: EXPLORING THEIR COLLECTIVE BEHAVIOR

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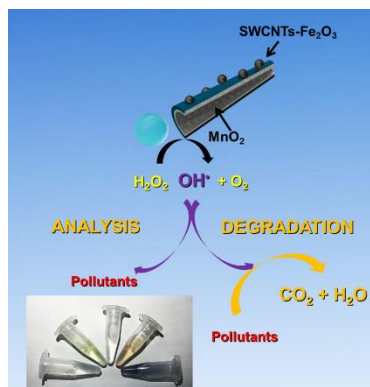
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Micromotors represent one of the most exciting horizons in micro and nanotechnologies, due to their great potential for a plethora of relevant applications in biosensing and environmental remediation. The utilization of self-propelled micromotors in (bio)chemical assays has led to a fundamentally new approach where their continuous movement around the sample and the mixing associated effect, all this as a collective behavior, greatly enhances the target-receptor interactions and hence the performance of the assay or the efficiency of removal or degradation of environmental pollutants [1, 2].

Micromotors are constituted by a few microscale layers that confer them self-propulsion (catalytic layer, *e.g.*, platinum nanoparticles, PtNPs, magnesium for water-based propulsion, MnO<sub>2</sub>), (bio)functionalization capabilities (sensing layer, *e.g.*, graphene oxide, rGO, carbon nanotubes), and magnetic guidance (magnetic layer, *e.g.*, Ni or ferrite nanoparticles). In addition to integrating nanomaterials, micromotors technology can also incorporate molecular recognition-based functionalization (*e.g.*, aptamers, antibodies, enzymes) and they are highly compatible with electrochemical and optical detection approaches, and even with microfluidics. In addition, in recent years, autonomous micromotors have also been used as sustainable and efficient micro cleaners to remove pollutants in water samples, and other related applications (see Figure 1).

In this plenary conference, formally speaking, the analytical possibilities of micromotors for the *on-the-fly* (bio)sensing and environmental remediation applications will be discussed.



**Figure 1.** Single-walled carbon nanotubes (SWCNTs) ferrite micromotors based on MnO<sub>2</sub> catalyst for sensing and degradation of pollutants.

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### Acknowledgements

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# **Premio Giovane Ricercatore**

## ENZYME-BASED AMPEROMETRIC BIOSENSORS: 60 YEARS LATER...QUO VADIS?

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Current research on enzyme based amperometric biosensors deals essentially with the same target analytes as was at focus in the early days of biosensor research, that are those within the clinical/medical, food/agriculture, and environmental fields. However, there has been substantial progress through the years and progress continues [1].

Communication between a redox enzyme and an electrode has been a central theme and continues along the traditional three major electron transfer (ET) routes, that are 1<sup>st</sup>, 2<sup>nd</sup> (mediated electron transfer) and 3<sup>rd</sup> generation biosensors (direct electron transfer, DET). DET consists in the direct electronic connection between the redox center of the enzyme and the electrode surface, which is working as a signal transducer. DET has been the target for many investigations both as a scientific challenge but also for practical reasons as a DET approach would simplify the construction of a biosensor and minimize the influence of other possible interfering components in the sample as well as allow mechanistic studies of the enzyme [2].

Beyond DET, another important topic within enzyme based amperometric biosensors is the possibility to target analytes that are not involved in ET chains by using chimeric/allosteric enzymes [3,4].

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# Keynotes

**EFFECTIVE DEGRADATION OF IBUPROFEN THROUGH AN ELECTRO-FENTON PROCESS IN THE PRESENCE OF ZERO-VALENT IRON**

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Considerable scientific interest has arose recently about the so-called contaminants of emerging concern (CECs) because these compounds are recalcitrant to biodegradation, and too hydrophilic to be easily partitioned on solid adsorption phases, or eliminated from water with the spent bacterial sludge.[1] Therefore, CECs are often released by traditional wastewater treatment plants into surface-water acceptor bodies, where they can produce harmful effects to living organisms. [2] Numerous technologies (denoted as Advanced Oxidation Processes) have been proposed for the production of highly reactive species able to completely degrade CECs.

Among the proposed AOPs, the Fenton and Fenton-like processes are promising techniques for the removal of highly recalcitrant contaminants.[3] The optimization of the processes and the comprehension of the operational mechanisms (e.g. the identification of the most important reactive species and the transformation pathways for the degraded compounds) are challenging. The classic problem solving approach of the analytical chemistry and the specific tools of “our” discipline (e.g. speciation study, identification and quantification of the main species...) are essential to give insights into the complexity of the Fenton and Fenton-like processes.

As an example, modified ZVI-Fenton with gradual and *in-situ* electrochemical generation of H<sub>2</sub>O<sub>2</sub> has been investigated to remove the non-steroidal, anti-inflammatory drug ibuprofen and to avoid •OH scavenging by a single large dose of H<sub>2</sub>O<sub>2</sub>. [4] ZVI-electro-Fenton achieved complete degradation under a variety of conditions, including pH 6 (more compatible with water treatment, than the more acidic pH values often needed by Fenton processes). A correct ratio between ZVI loading and H<sub>2</sub>O<sub>2</sub> production rate (electrochemically controlled) is fundamental for ibuprofen degradation. Low ZVI loading coupled with the application of square-wave potential achieved good performance in the degradation of ibuprofen, with considerable resource economy in terms of materials and energy supply.

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**A MULTI-ANALYTICAL APPROACH BASED ON MALDI-MS AND SPECTROSCOPIC TECHNIQUES FOR THE IDENTIFICATION OF SPRAY PAINTS USED IN URBAN ARTWORKS**

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Street art has become one of the most appreciated cultural movements of our time as proved by the intensification of the street art market and the organization of committed indoor and open-air museums and exhibitions. Even though the necessity to preserve urban contemporary art is commonly accepted in terms of protecting outdoor murals and removing defacing graffiti from artworks, the conservation strategies are still questioned. The contemporary spray paint materials used in urban artworks contain various binding media, additives, and synthetic pigments, susceptible to degradation under the influence of environmental conditions such as light irradiation, temperature, and pollutants, posing tasks only recently faced by conservators. Identifying and detailing materials is crucial to select conservation strategies including cleaning, protective coatings, and removing unwanted graffiti.

Matrix-assisted laser desorption/ionization with time-of-flight mass spectrometry (MALDI-ToF-MS) is very suitable for polymer analysis since the soft ionization process prevents the oligomers' fragmentation and their detection as singly charged chains [1]. However, the application of MALDI-ToF-MS to polymeric binders in modern spray paints is limited probably because of the complexity of such formulations. Most likely, a multi-technique approach including spectroscopic techniques is required to characterize binders and/or pigments in spray paints. Some outcomes here presented will demonstrate how the classification of spray paints is challenging [3] in binding media recognition. This work was supported by Progetto di Ricerca di Interesse Nazionale—PRIN 2020MNZ579—“Sustainable Preservation Strategies for Street Art - SuPerStAr”, financed by the Italian Ministero per l'Istruzione, l'Università e la Ricerca (MIUR).

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**THE MONITORING OF MICROPLASTICS IN COMPLEX AQUATIC MATRICES: THE POTENTIAL OF NEAR INFRARED HYPERSPECTRAL IMAGING (NIR-HSI) COMBINED WITH CHEMOMETRIC STRATEGIES**

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Due to the enormous production and mismanagement, the issue of plastic waste represents a critical and serious social, economic and environmental challenge. Once in the environment, plastic wastes can be degraded by physical, chemical and biological factors (e.g., ultraviolet radiation, wind or water erosion, microorganisms) becoming small plastic debris characterized by reduced dimensions (between 1 µm and 5 mm) and commonly referred to as microplastics (MP). As the marine system is the final sink for MP, there is an urgent need for developing reliable methods enabling to monitor the presence of synthetic particles in different marine compartments and sample matrices. To date, one of the major constraints and challenges in MP research is to conduct extensive environmental monitoring using an adequate number of replicated samples to detect relevant spatial and temporal variations in MP abundance and distribution patterns. Furthermore, the lack of methodological harmonisation in traditional analytical procedures, applied for MP detection, does not allow comparability among data to be achieved from different studies and research laboratories. So far, different analytical methods have been proposed to detect MP in field samples. The most used methods are based on the visual sorting of MP followed by spectroscopic single-point techniques. However, all the traditional spectroscopic methods present common limitations: they are usually time and/or cost consuming, may require extensive sample manipulations and their sensitivity and selectivity strongly depend either on the initial visual pre-sorting of MP or on the number of samples that can be analysed.

To date, the potentialities of near infrared hyperspectral imaging (NIR-HSI) systems are still poorly investigated and limitedly used to properly address real ecological and analytical issues related to the detection of MP.

Indeed, NIR-HSI represents a potential cost and time effective method, enabling a direct and fast (few minutes for the analysis of an entire filter of 5 cm diameter) detection of MP without heavy sample purification and manipulations, thus avoiding potential procedural bias related to particle pre-sorting steps. Moreover, the potential of the method can be significantly amplified and made effective thanks to the combination with multivariate data processing methods, in order to extract useful information from the complex 3D data array. The automated normalised difference image (NDI) strategy, which permits to sensibly reduce the time needed for the processing and the evaluation of data will be presented.

Thanks to the time and cost effectiveness, a large-scale implementation of this method would enable to extensively monitor the MP presence in natural environments for assessing the ecological risk related to MP contamination.

**ENANTIOSELECTIVE LIQUID CHROMATOGRAPHY ANALYSIS OF AMINO ACIDS UNDER ECO-FRIENDLY CONDITIONS**

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Natural and unnatural amino acids (AAs) are privileged building blocks in drug design, while the conserved backbones and their variable side chains are of relevance in a wide number of other domains, which span from probing protein function to adhesive agents and biomedical products, to mention but a few. Moreover, the discovery of a multitude of naturally occurring bioactive peptides has fuelled the interest towards the synthesis of peptidomimetics made up with unnatural AAs [1]. Still, a body of evidence strongly suggests that some essential AAs are often scarcely assumed by diet, which justifies the progressive increase in the consumption of food supplements labelled to contain only L-AAs. Also notably, many evidences have shown that D-AAs are ubiquitously present in many organism including humans and fulfil specific biological functions, while their presence food products can denote thermal and alkaline treatments as well as microbial contamination [2]. On the basis of the above, to avail of methods enabling the correct determination of the enantiomeric composition of AAs is of prior importance. The enantioselective LC analysis of AAs can be performed through either direct or indirect methods. Direct methods are based on the separation of underivatized AA diastereoisomers on chiral stationary phases (CSPs) or with a achiral stationary phases used in combination with chiral eluents. Indirect methods are often subjected to issues and require specific and sometimes long and environmentally unfriendly derivatization procedures with chiral derivatizing agents. On the contrary, the application of direct methods with CSPs help to overcome most of the drawbacks inherent to the indirect approach, with the additional possible advantage to use environmentally sustainable mobile phases [3]. Indeed, enantioselective LC analyses of AAs can be efficiently performed under both RP and PO/PI conditions with the most relevant CSPs carrying crown ethers, cyclodextrins, glycopeptides, *Cinchona* alkaloids, polysaccharides and proteins type chiral selectors. Also noteworthy is the possibility with these CSPs to use mobile phases compatible with MS detectors, thereby opening the way to accurate analysis of complex samples. All these points will be treated in the lecture, also highlighting the contribution by *in silico* studies to the greenness of the process as a whole.

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**HIGH RESOLUTION MASS SPECTROMETRY: ANALYTICAL CHALLENGES AND NEW OPPORTUNITIES**

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Recently high resolution mass spectrometry (HRMS) has advanced as the most powerful tool for the unambiguous determination of compounds in samples of food, environmental and toxicological concern [1,2]. The advantages provided by HRMS including the possibility of retrospective data analysis and the ability to perform structural elucidations of unknown or suspected compounds compared to classical tandem MS with unit mass resolution are considerable. Taking into account that pre-treatment could affect sample integrity, the coupling of ambient ionization sources with HRMS has gained wider acceptance in the last few years for direct characterization of different compounds with minimal or no sample preparation, thus avoiding chromatographic separation, and reducing sample handling, costs and analysis time [3,4]. Being able to provide a comprehensive fingerprinting of the investigated samples as well as the identification of new markers, HRMS-based omics strategies are particularly challenging because of their sensitivity, high-throughput and discriminating power. Ion mobility mass spectrometry is an additional feature proposed in a broad range of applications because of its ability to provide increased separation space, removal of chemical noise, and structural insights. Finally, MS imaging approaches can provide a unique combination of molecular and spatial information of a wide variety of compounds in complex biological samples. The main benefits of identification strategies using different mass analyzers will be highlighted with a focus on topics related to food safety and quality assessment, forensics and toxicological investigations. Finally, the fundamental role of chemometrics for data processing will be critically discussed.

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**A NEW SYSTEM BASED ON REAL-TIME ELECTRON IONIZATION MASS SPECTROMETRY (REI-MS): DEVELOPMENT AND APPLICATIONS**

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Mass spectrometry (MS), by itself or coupled with chromatography, is the most powerful analytical technique. The latest advances in liquid chromatography (LC) coupled with MS are aimed at improving its identification reliability, for instance through the application of the linear retention index system [1] or, more incisively, by the development of the liquid electron ionization (LEI) interface [2]. LEI allows the vaporization of analytes in the liquid phase at atmospheric pressure inside a specific microchannel, before entering into the electron ionization (EI) source where they are ionized generating library searchable spectra. Most recently LEI was successfully applied to introduce a liquid flow into a chemical ionization (CI) source [3].

Ambient mass spectrometry (AMS) is nowadays a rather established technique even if atmospheric pressure ionization compromises its identification power [4]. The same principle of LEI was applied to real-time electron ionization mass spectrometry (REI-MS), a new technique designed to combine the advantages of AMS with EI. The current REI configuration consists of an EI source coupled to a single quadrupole mass spectrometer which allows the aspiration of the sample through a fused silica capillary (30 µm I.D., 375 µm O.D.), thanks to the vacuum it generates. A syringe pump allows the solvent delivery through a co-axial capillary, placed around the aspiration one, whereas an open/close valve interrupts the aspiration during stand-by. Analytes entering the ion source in a liquid phase are sprayed into the ion source, vaporized, and ionized by EI. Preliminary data are very encouraging. REI was successfully applied for targeted, untargeted, and 2D analysis in several matrices: fruit peels treated with pesticides, medicinal tablets, banknotes imaged with cocaine spots, and paintings. More configurations and instrument setups will be tested on several matrices, to increase REI application fields, and also in the view to realize a portable device. Green assessment tools demonstrated the environmentally friendly character of this approach.

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**HIGH SENSITIVITY vs SIMPLICITY: DETECTION STRATEGIES FOR LATERAL FLOW ASSAY**

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The lateral flow assay (LFA) technique has gained great attention recently for point-of-care testing, especially in the clinical field. More generally, the method allows the development of rapid, sensitive and specific biosensors, to detect very diverse compounds (from chemical contaminants to virus) in complex matrices (biological fluids, food extracts, and so on), and for various applications [1]. As such, it has been impressively developed and improved in the last few years, in particular by combining the principles of bioassays to high sensitivity detection strategies.

In principle, LFA uses porous materials to transport the sample and reagents along a two-dimension support, where selective and high affinity bioligands are immobilized in spatially confined zones. The reactions occurring in reactive zones depend on the presence and amount of the target analyte, and are revealed by some probes, which typically is composed of a recognition element and of a signal reporter. Several signal reporters can be employed for developing fast, cheap, and user-friendly devices, based on colorimetric (visual) detection. Nanomaterials, such as noble metal and carbon nanoparticles, have been conveniently used for the purpose. To overcome limitations of LFA sensitivity, other detection strategies have been proposed as well, such as those based on luminescence phenomena and surface enhanced Raman spectroscopy (SERS) [2].

In this presentation, an overview of different detection strategies for LFAs, illustrated by applications developed by our research group in collaboration with several partners, will be presented and discussed. The examples will include colorimetric, luminescence, SERS-based, and electrochemical detection, as a novel, highly sensitive and affordable detection strategy to design next generation LFA-based biosensors.

The different approaches will be critically discussed considering how they address the inherent challenges of point-of-care (POC) diagnostics, such as lowering detection limits, multiplexing and quantification of analytes in complex samples. The future perspective and the expected impact of combining new detection strategies to the LFA platform will be suggested.

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## THE “CELL CHROMATOGRAPH”

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Advanced Therapy Medicinal Products (ATMPs) today are used for gene&cell therapy, and tissue-engineered medicines. Cell-based ATMPs are drugs altogether. As for standard drugs, technologies for quality control, and non-invasive isolation and production of cell-based ATMPs are then needed to ensure their rapidly expanding applications and ameliorate safety and standardization of cell production. Nonetheless, effectiveness of the existing techniques for the isolation, characterization, and Quality Control (QC) of cell-based ATMPs is still insufficient.

Over more than three decades, we have developed and transferred Field-Flow Fractionation (FFF) methods and technologies for bio-analytical characterization of nano-sized and micro-sized particles. From an indirect derivation of FFF, we have invented Non-Equilibrium Earth-Gravity Assisted Dynamic Fractionation (NEEGA-DF) [1]. We then implemented NEEGA-DF into our designed, developed, engineered, and finally industrialized *cell chromatograph* Celector® [2]. Analogously to a liquid chromatograph, Celector® separates, characterizes, and sub-fractionates living cells from even complex samples, for further, downstream cell QC, characterization, and/or culture.

Stem cells (SCs) are among most used ATMPs to regenerate damaged tissues and organs. They are also increasingly used by pharmaceutical companies for screening novel drugs. SC-based ATMPs for pets are also booming. The global SC therapy market is expected to reach ca. €48B by 2027. We have shown Celector® able to select, without extra manipulation, the most potent cell components from heterogeneous SC populations of different origin [3-9]. Celector® can be then used to obtain homogeneous and “good” cell-based ATMPs even from very heterogeneous cell samples to improve the success rate of ATMP applications in regenerative medicine.

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**THE ROLE, THE BEHAVIOUR AND THE SPECIATION OF THE METAL CATIONS IN MIMETIC SYSTEMS**

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Approximately two-thirds of the elements in the periodic table can be labelled as metals. The role of the metals is polyvalent, essential or toxic, depending on the concentration and on the form (free or complexed metal) in which is present in a generic system as a biologic fluid (plasma [1], saliva [2], urine), natural water (sea water [3]) and wastewater.

To focus and fully characterize the behaviour of metals in aqueous solution, it is necessary to consider and evaluate the effect of variables such as pH value, ionic medium, ionic strength, temperature, concentration of the metal, eventually presence of the inorganic and/or organic secondary ligand, that they can affect the speciation, distribution and availability of a metal cation.

The recovery and processing of the metals from waste materials is an integral part of the economies, as well as the metals are essential in several indispensable biochemical processes for living organisms. For example, transition metal complexes are important in catalysis, materials synthesis, photochemistry, biological systems and for medicinal purposes as therapeutic compounds and/or agent.

Water is without a doubt the most common ligand and the hydrolysis reactions occur for closely all the metal cations and profoundly affect their chemistry. These reactions determine the form of metal in solutions as well as their charge and stability.

Hydrolysed metal salts, as of aluminium or iron, are widely employed as coagulants in water treatment. They have a fundamental role in the removal of many impurities from polluted waters. These impurities include inorganic particles, such as clays, pathogenic microbes, and dissolved natural organic matter. The different role and behaviour of one metal is directly connected to its concentration and its speciation, therefore it is of fundamental importance to know the equilibria that are established in a specific chemical system.

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**DO WE REALLY NEED NANOMATERIALS IN THE FIGHTING AGAINST SARS-CoV-2? THE OPINION OF AN ANALYTICAL SPECTROSCOPIST**

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The development of nanoantimicrobials (NAMs) has been the focus of our research in the last decade: we offered a number of technological solutions for the production of active nanoparticles (NPs) and composite materials to fight antimicrobial resistance. A thorough analytical characterization of NAMs demonstrated that they exert a controlled release of bioactive ions without releasing intact NPs of potential toxicity. In 2019, the new severe acute respiratory syndrome coronavirus (SARS-CoV-2) started spreading around the world. Since then, many strategies for its prevention and control have been studied and implemented. One of the characteristics sought in a (nano)antiviral treatment is to ensure the cleaning and sterilization of common touch, inanimate surfaces, especially in all those contexts where contagion could run fast: hospitals, schools, transports, and public places [1]. Here we report on the efficacy of electrosynthesized ZnONPs against SARS-CoV-2. NPs have been produced by a scalable and ecofriendly method [2], based on a electrochemical sol-gel approach. The peculiarities of this route to metal oxide nanoantimicrobials will be discussed and compared to alternative methods. The results of microscopy (TEM), diffraction (SAED) and spectroscopy (UV-Vis, ATR-IR, XPS) analyses will be argued, offering an overview of the materials structure, composition and surface reactivity. The NP antiviral action has been tested in vitro against SARS-CoV-2, through chemiluminescence assays, showing a decrease of the viral loading up to 100%, as a function of the material composition [3]. Usage of these nanoantimicrobials as coatings with controlled ionic release, for hard touching surfaces, is envisaged. Application of our NPs as antibacterial/antibiofilm agents in other technological fields will be concisely overviewed, and conclusions will be drawn about the most exciting perspectives in the field of nanoantimicrobials.

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**CHEMOMETRIC APPROACHES IN METABOLOMICS: AN OVERVIEW DESCRIBING A REAL CASEWORK FOR THE DEVELOPMENT OF AN EFFECTIVE TEST FOR BREAST CANCER SCREENING**

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Metabolomics is a comprehensive analysis of metabolites produced by a biological system or derived from various other external sources, whose analysis of which is divided into targeted and untargeted approaches. A typical metabolomics workflow begins with collecting biological samples to answer specific research questions. The collected datasets typically contain hundreds to thousands of variables derived from multiple sources of variability; therefore, improper data analysis can lead to inaccurate identification of metabolites, poor interpretive models, and compromise the efficiency of the applied data analysis procedures. Particular emphasis is placed on the pre-processing required to interpret data from mass spectrometry (MS) (in combination with gas chromatography (GC) or liquid chromatography (LC)), which includes steps such as peak detection and integration, retention and migration time alignment, and gap-filling. Subsequently, chemometric approaches simplify the complexity of high-dimensional data into manageable variables, allowing straightforward and meaningful interpretation of the large complex metabolomics datasets for classification or regression purposes. In the present study, we present a practical case involving targeted and untargeted UHPLC-QTOF-MS /MS analyses of urine samples to develop an effective test for breast cancer screening in postmenopausal women. To achieve this goal, supervised classification models (such as Partial Least Squares Discriminant Analysis (PLS-DA) and Random Forest (RF)) were used to discriminate individuals positive for tumour pathology from healthy individuals. This project's future goals envision applying these models to the untargeted data for clinical and diagnostic testing or follow-up, with the possibility of developing stratification models using only the data from the cohort with positive cancer pathology.

**NOT ONLY ELECTROANALYSIS: APPLICATIONS OF PRUSSIAN BLUE DEVELOPING COLORIMETRIC (BIO)SENSORS**

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The search for analytical strategies to ensuring clinical monitoring, without recurring the use of laboratory and skilled personnel, is highly required. However, the development of brand-new Point-of-Care devices cannot overlook the successful combination among manufacture methodologies, functional (smart)materials, and transduction mechanisms. In particular, the era of sustainability we belong in, strictly requires user-friendly and low-cost approaches, adaptable in low-resource contexts, i.e. developing world. Among the different sources of sensing materials, Prussian Blue is also emerging as a colorimetric probe, although it is largely used as electrocatalyst for hydrogen peroxide and thiols detection. Despite all the approaches reported to date, we decided to highlight the use of Prussian Blue in order to realize a novel class of analytical tools. In particular, two examples will be presented: the detection of sweat urea through the degradation of Prussian Blue due to the alkaline pH produced by the urease enzyme, and the speciation of Fe(II) and Fe(III) following the formation of Prussian Blue starting from two precursors, respectively, Ferrocyanide and Ferricyanide. The keynote presentation will provide the audience a wide perspective highlighting the different synthetic routes, applications and challenges around the world of Prussian Blue. Two systems will be majorly described as the latest achievements of the research group, demonstrating the possibility to produce assured paper-based platforms for the use of non-specialists, demonstrating optimistic outputs.

**PAPER-BASED ELECTROCHEMICAL (BIO)SENSORS AS SMART AND SUSTAINABLE DEVICES**

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In the last decade, electrochemical paper-based (bio)sensors have garnered much attention in the sensing field thanks to their cost-effectiveness, easiness to use, and miniaturization. Besides these characteristics in common with the other electrochemical (bio)sensors, the features of paper such as foldability and porosity have opened new unprecedented electrochemical (bio)sensor configurations allowing for reagent-free measurements, origami-like set-up, and the absence of sample treatment. Furthermore, paper-based electrochemical devices have overcome the limitation of other electrochemical sensors, being able to detect the target analytes not only in solution but also in aerosol phase and surface without any additional instrument, matching one of the top 10 emerging technologies of 2021, namely diagnosing diseases with a puff of breath. In addition, after the measure, the device can be burned reducing waste management with a relevant decrease of analysis costs in the case of biological fluids. In this keynote lecture, I will report how we have exploited the features of paper to design smart electrochemical biosensors able to treat the sample, to contain any reagent needed for the measurement, to on-site synthesize nanomaterials, and to make the measurement delivering novel paper-based devices applied in the biomedical, defense, environmental, and agrifood sectors.

## UNLOCKING THE FUTURE OF COMPREHENSIVE TWO-DIMENSIONAL CHROMATOGRAPHY IN FOOD-OMICS BY ARTIFICIAL INTELLIGENCE ALGORITHMS

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Since its introduction, comprehensive two-dimensional gas chromatography (GC×GC), has unveiled its potentials in many fields helping scientists to better understand the Nature’s complexity, facilitating highly-informative screenings, supporting markers discovery in omics applications and offering many opportunities to implement system biology-like strategies for investigation, the so-called *integrationist* approach [1].

In food “omics” the analytical platform design and configuration plays a key role to achieve the suitable information capacity, resolution and sensitivity to answer the many questions posed by application needs.

The contribution deals with the challenging task of designing a multidimensional platform for food metabolomics [2] implemented by an effective data processing workflow. A strategy capable to answer many questions about product qualities (e.g., sensory quality, freshness, authenticity, presence of sensory defects etc.) with a single measure realized by combining many analytical dimensions (e.g., sample preparation, separation, multiple detection, olfactometry, etc.).

Within this context, the key-role of Artificial Intelligence (AI) algorithms for *computer vision* (i.e., “...a field of AI that enables computers and systems to derive meaningful information from digital images...” [3]) and *smelling* (e.g., AI smelling machine [4]) is discussed and proof-of-evidence on the feasibility and effectiveness of such “comprehensive” approaches presented through the authors research experience on high-quality extra-virgin olive oil.

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**THE CHALLENGING SPECIATION OF Cr(VI) IN TOXICOLOGICAL RELEVANT MATRICES: WHERE OFFICIAL METHODS CAN FAIL AND HOW TO DEVELOP ALTERNATIVE PROTOCOLS**

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Chromium speciation is an extensively studied topic owing to the striking diverse toxicity of its two most stable oxidation states, i.e., Cr(III) and Cr(VI) forms [1]. The former is considered an essential nutrient for living organisms with a low toxicity profile, while the second is classified as carcinogenic to humans. To perform a reliable risk assessment against chromium exposure, it is therefore necessary to accurately quantify Cr(VI), avoiding any Cr(III)/Cr(VI) interconversion during the overall analytical protocol, as only Cr(VI) exposure is relevant. Due to large variety of matrixes where it is important to assess Cr(VI) concentration (waters, foods, soils, airborne particulate, etc.), a consequent large variety of methods was developed, with new methods still under development to solve the problems of existing ones. Wanting to generalize, major issues are the unpredictable role of coextracted substances in the Cr(III)/Cr(VI) equilibrium, together with the presence of high Cr(III) concentrations. A typical example of problems encountered with official methods could be the determination of Cr(VI) in Cr(III)-rich particulate matter: such methods involve a hot extraction in extremely basic medium which systematically oxidizes a non-negligible fraction of Cr(III). These methods can be therefore used in all cases where the Cr(III)/Cr(VI) ratio is quite low, as the overestimation of Cr(VI) could lie within the experimental error. From an analytical chemists' point of view the presence of high Cr(III) concentrations certainly represent a challenge, also because the role of coextracted substances (which is enhanced at high Cr(III)/Cr(VI) ratios) cannot be predicted on a sample-by-sample basis.

In this key note the development of a strategy which enables the detection of problems during the determination of Cr(VI) with various methods is presented. The case study of the comparison of official and newly developed methods for the determination of Cr(VI) in particulate matter will be presented, too, pointing out where the official method fails and how to develop an alternative protocol.

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**ADVANCED ANALYTICAL TOOLS BASED ON COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY COUPLED WITH MASS SPECTROMETRY FOR THE ANALYSIS OF BIOMASS AND PLASTIC WASTE SAMPLES**

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The use of comprehensive two-dimensional gas chromatography (GC×GC) coupled with mass spectrometry (MS) has been widely exploited in the field of petroleomics and related samples. In this contribution, this analytical methodology has been successfully applied to investigate volatile and semi-volatile compounds of biomass feedstock and plastic waste. On one hand, to facilitate a possible transition from crude oil to biofuels production and, on the other hand, to improve plastic recycling, limit plastic pollution, and promote the circular economy.

Biomass feedstocks are among promising candidates to possibly fulfill requirements as substitutes for crude oils as primary sources of chemical energy feedstock. In this context, the use of animal fats could be an interesting alternative for biodiesel production as the production of animal byproducts represents nearly 17 million tons per year in the European Union only (2020). Animal fats, however, contain large amounts of non-esterified fatty acids and other oxygen compounds, reducing the yield of biodiesel. Therefore, specific pretreatments are often needed before the trans-esterification process.

On the other hand, plastic production worldwide is expected to continue to grow every year and plastic waste and its reuse are a current issue in which industries are focusing on limiting its production. Indeed, according to the annual production of plastics worldwide, in 2020 about 370 million tons of plastic were produced in the world. Chemical recycling, particularly pyrolysis of plastic wastes, could be a valuable solution to resolve these problems and provide an alternative pathway to produce “recycled” chemical products for the petrochemical industry. Nevertheless, the pyrolysis oils need a deep

investigation before the upgrading test to re-use them to generate new recycled products.

In the light of this consideration, a detailed characterization of major and minor components of these samples is of primary importance to support the development of dedicated processing.

Different analytical strategies and analytical platforms using mainly GC×GC coupled with both LR- and HR-TOFMS are applied for biomass feedstock and plastic waste characterization. The use of liquid chromatography (LC) as a pre-separation step before GC analysis can be considered an additional tool to isolate the fractions of interest highlighting the otherwise “hidden information” of minor components present in the samples.

**PROTEIN RECOGNITIVE SOFT MOLECULARLY IMPRINTED NANOPARTICLES FOR PLASMONIC SENSING PLATFORMS**

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Soft, deformable, molecularly imprinted nanoparticles (nanoMIPs), that are tailor made biomimetic receptors, prepared by means of a template assisted synthesis [1], were combined to nano-plasmonic platforms to develop highly sensitive bio/chemical sensors.

Soft nanoMIPs ( $d_{\text{mean}} < 50$  nm) were prepared to selectively bind a target protein, Bovine Serum Albumin (BSA), and used to functionalize optical nanostructures placed on a polymethyl methacrylate substrate that was exploited as slab waveguide. The behaviour of nanoMIP-functionalized gold nanogratings with on periodic nano-stripes, or of nanogratings with a deposited ultra-thin MIP layer ( $< 100$  nm) or of nanogratings functionalized with antibodies were compared. The sensors performances were tested by the detection of BSA using the same setup, in which the periodic nano-stripes allocated in a longitudinal orientation with respect to the direction of the input light.

Result demonstrated the nanoMIP-nanogratings showed superior performance with respect to the ultra-thin MIP-layer nanogratings. The peculiar deformable character of the nano-MIPs enabled to significantly enhance the limit of detection (LOD) of the plasmonic bio/sensor, allowing the detection of the low femtomolar concentrations of analyte (LOD  $\sim 3$  fM), thus outpassing of 4 orders of magnitude the sensitivities achieved so far on optimized nano-patterned plasmonic platforms functionalized with ultra-thin MIP layers. Thus, the soft nanoMIPs onto plasmonic probes permit to attain ultralow detections, down to the quasi-single molecule. As a general consideration, the combination of more plasmonic transducers to different kinds of MIP receptors is discussed as a mean to attain the detection range for the selected application field.

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# **Oral Communications**

**IDENTIFICATION OF MARKER PEPTIDES OF SPIRULINA ALLERGENS BY LC-ESI-MS/MS**

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Novel foods are all foodstuffs or food ingredients never used for human consumption in the EU before 15 May 1997. This definition mainly encompasses exotic foods, such as algae, microorganisms, and insects. Algae, especially microalgae such as spirulina (*Arthrospira platensis*) are largely used as food supplements worldwide for their health benefits related to the high protein and vitamin contents, polyunsaturated fatty acid ( $\omega$ -3 and  $\omega$ -6), bioactive peptides, and so on [1]. Today, few studies have focused on the potential allergenicity of novel foods, such as spirulina, that could trigger side effects in sensitized people causing serious health problems. Very recently, we have developed an innovative strategy [2] to search for known and putative allergenic proteins in the microalgae spirulina and chlorella according to WHO/FAO guidelines. In this communication, the identification and quantitation of spirulina putative allergenic proteins both in food supplements and in processed foodstuffs are presented and discussed [3]. The determination of marker peptides was performed by reversed-phase liquid chromatography with high-resolution tandem mass spectrometry. Three marker peptides were identified for C-phycoerythrin beta subunit, a known spirulina allergenic protein. Specifically, ETYLALGTPGSSVAVGVGK and YVTYAVFAGDASVLEDR were chosen as qualifier peptides and ITSNASTIVSNAAR as a quantifier peptide. Their chemical and thermal stability was assessed by homemade spiked and incurred samples, *i.e.*, cookies and fruit juice. Linearity, limits of detection/quantification, recovery, repeatability, reproducibility, matrix, and processing effects were evaluated for method validation.

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**ANALYTICAL COMPOSITION OF FLOURS THROUGH THERMOGRAVIMETRIC AND RHEOLOGICAL COMBINED METHODS**

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Thermogravimetric analysis (TGA) and rheology are two analytical methods that can provide information on the interaction between flour components and water helping to characterize the dough. To our knowledge few studies have been conducted to predict analytical composition of flour from thermogravimetric and rheological measurements. The main chemical components of flour are usually quantified by Approved Methods of Analysis AACC methods, that, to obtain flour characterization as a whole, takes a considerable amount of time and a great amount of sample since each individual method provides data about a single parameter. In our study the suitability of thermogravimetric analysis (TGA) and rheology for predicting analytical composition of flour was verified. The percentage weight loss of flour samples in different temperature ranges and their analytical composition determined following the American Association of Cereal Chemists (AACC) approved methods were correlated. The moisture content determined by AACC method showed a strong correlation with the weight loss of dried samples in the temperature range 30 – 125 °C. Similarly, the weight loss of fully swollen samples, determined by TGA in the temperature range of 30 – 125°C, perfectly correlated with the water-Solvent Retention Capacity (w-SRC) determined by standard method (AACC). The weight loss in the range 200 –400 °C revealed a good correlation with the starch content, while the weight loss in the range 400 – 600 °C correlated with the gluten content. Finally, the so-called residue at 600°C (usually attributable to mineral content) correlated with the ash content (AACC standard method). The rheological parameters ( $G'$ ,  $G''$  and  $\tan \delta$ ) also showed a strong correlation with the analytical composition of analysed flours. A good correlation was observed between the mineral content and the elastic modulus, as well as between both  $G'$  and  $G''$  and the starch content. As the resilience of dough samples strongly depends on the relative amount of gliadins and glutenins, a strong correlation between the Gli/Glu ratio and  $\tan \delta$  was also found [1].

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**ECOLOGICAL TRANSITION IN THE FIELD OF PACKAGING AND FOOD CONTACT MATERIALS: NEW TRENDS AND EVALUATION OF SAFETY AND STABILITY**

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The recent European Regulation on Single Use Plastic is challenging an entire productive sector that is proposing new solutions to move towards an ecological transition. Different new trends are emerging in the field of food contact materials and packaging. Unlikely, all solutions are accompanied by important drawbacks related to limited performances, lower quality, requirement of thicker layers, higher mass, and additives. Paper and wood are often proposed, but they need to be reinforced with coating and surface treatments. Use of recycled material is encouraged, despite a lack of infrastructures, energy requirement, and the lower quality of the recovered material. Biopolymers, often produced from agricultural sources, show limited barrier properties and low stability during ageing.

To overcome these limits, biobased composite materials can be produced from biopolymers reinforced with suitable fillers or nanofillers including natural compounds from agroindustrial byproducts. An example is the combination of a poly(butylene adipate-co-terephthalate) (PBAT) substrate with microparticles of a zein-titanium dioxide complex. Filaments suitable to be 3-dimensional printed by fused deposition were obtained.

In this context, the development of reliable analytical methods is mandatory to control natural resources used as substrate, and to assess performance and safety of new materials, as well as to evaluate their stability and shelf-life. The composition of biobased materials, and the additives used are usually unknown; besides there is not a clear protocol to follow for untargeted analysis and safety assessment. Therefore, the combination of multiple approaches based on the synergy of different analytical techniques such as chromatography, mass spectrometry, FTIR-ATR, has been exploited to obtain complementary information.

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## POMEGRANATE SEEDS: A HIGH INTEREST FOOD WASTE

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Pomegranate seeds represent a highly interesting waste. These come from the work-up of pomegranate fruit, both in terms of bagasse, coming by squeezing of whole fruits, and as cleaner residue, coming by centrifugation of arils, previously separated. The excellent composition of pomegranate fruit in all its parts, peels, arils and seeds, in terms of bioactive and healthy compounds, make the study and the valorization of these products of great interest for the scientific community, as established by the number of published papers on this topic [1,2].

The high volumes of commercialized pomegranate juices, already significant in Italy as well as in other Mediterranean countries, are constantly rising all around the world. So the recycling of this waste means not only reduction of residues, costs and environmental impacts but also transformation in co-products with healthy and other useful potentials.

Separated and dried whole seeds, gently gifted by a local producer of Campania region and belonging to the Dente di Cavallo cultivar, were powdered and submitted to different extraction procedures (classic, Soxhlet, supercritical CO<sub>2</sub>) with the aim to evaluate extraction yields and oil composition. Triglyceride oils were analyzed by <sup>1</sup>H-NMR, GC-MS and MALDI-TOF analysis. Their anti-radical potential was also evaluated by DPPH analysis and a correlation will be attempted with the adopted extraction methods and the contained antioxidant molecules.

In view of an approach of complete circular economy, the solid residues remaining after the first extraction step were furtherly submitted to extraction, in order to obtain and characterize the contained fibers, to be used as food supplements or as bio-packaging. Finally, a recycling procedure is under evaluation for the remaining parts, in order to achieve zero impact.

Preliminary results, coming from different powdering methods, different extraction methods and a multi-methodological analytical approach, show that the obtained oil (up to 15% of yield) is highly rich in punicic acid, an interesting healthy component. The exerted antioxidant activity seems to be influenced by the applied flow-chart.

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**THE COUPLING OF ENANTIO-SELECTIVE GAS CHROMATOGRAPHY TO ISOTOPIC RATIO MASS SPECTROMETRY: PERSPECTIVES AND THE NEED OF MULTIDIMENSIONAL GAS-CHROMATOGRAPHIC APPROACHES**

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Dealing with the analysis of natural expensive products, quali-quantitative data by conventional gas-chromatographic techniques, coupled to flame ionization detection and to mass spectrometry, are not always the answer for authenticity assessment. Comparable volatile profiles may be easily reconstituted by different sources, thus requiring additional analytical approaches to unveil adulterations. In this concern, enantio-selective gas chromatography (Es-GC) has been broadly recognized to tackle such an issue over the years. The detection of unusual enantiomeric excesses in commercial samples is often able to highlight adulterations, when compared to genuine reference data. As well as Es-GC, gas chromatography coupled to isotope ratio mass spectrometry ensures a clear distinction among plants having different photosynthetic cycles, as well as from synthetic sources. Since both the techniques investigate in depth the biochemical pathways of the plant, the coupling of a chiral separation to an isotope ratio mass spectrometry detection (Es-GC-C-IRMS) would ideally allow a simultaneous detection of both the parameters. However, several issues arise dealing with a monodimensional chiral separation. Although the chosen chiral column can resolve target enantiomers each other, co-elutions may involve with adjacent achiral components, strongly affecting the reliability of both the measurements. This issue is even more limiting for IRMS detection, due to the uneven retention of carbon isotopes along an entire peak of CO<sub>2</sub>, which would lead to wrong measurements in case of co-elutions. It is clear that such conditions require more sophisticated gas chromatographic approaches. In this research, multidimensional gas-chromatography (MDGC) is demonstrated to provide an improved resolution prior to parallel MS and IRMS detections. This system allowed to resolve most of the co-elutions for target volatiles, evident in monodimensional conditions

**RELEASE OF SELECTED NON-INTENTIONALLY ADDED SUBSTANCES (NIAS) FROM PET FOOD CONTACT MATERIALS: A NEW ONLINE SPE-UHPLC-MS/MS MULTIRESIDUE METHOD.**

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Food Contact Materials (FCMs) are an underestimated source of food chemical contaminants and a potentially relevant route of human exposure to chemicals that are harmful to the endocrine system. Foods and water are the main source of exposure due to the contact with the packaging materials, often of polymeric nature. Additives are added as dispersion in the polymeric matrix in small amounts to: *i*) improve the physical-chemical properties of the final product; *ii*) prevent the thermal oxidation; *iii*) avoid reticulation reactions of macromolecules chain [1, 2]. Many additives are considered endocrine-disrupting chemicals (EDCs) [3].

European Regulation 10/2011 requires migration tests on FCMs and foodstuffs to evaluate the presence of listed substances and of not intentionally added substances (NIAS) not listed in the regulation and not subjected to restrictions. NIAS are a heterogeneous group of substances classified with a potential estrogenic or androgenic activity. Subsequently the evaluation of the presence of these molecules in foods and water is significant. Here we presented an online SPE /UHPLC-tandem MS method to quantify trace levels of NIAS in food simulants (A: aqueous 3% acetic acid; B: aqueous 20% ethanol) contained in PET preformed bottles. The use of online SPE reduced the systemic errors thank to the automation of the technique. For the developed analytical method, we evaluated limit of detection (LOD), limit of quantitation (LOQ), selectivity, RSD% and BIAS% for LLOQ for a total of twelve NIAS, including monomers, antioxidants, UV-filters and additives. LOD ranged between 0.002 µg/L for bisphenol S and 13.6 µg/L for 2,6-di-tert-butyl-4-methylphenol (BHT). LOQs was comprised between 0.01 µg/L for bisphenol S and 42.2 µg/L for BHT. The online-SPE/UHPLC-tandem MS method was applied to the food simulants contained in several types of PET packaging materials to evaluate the migration of the selected NIAS. The results showed the presence (µg/L) of NIAS in the tested samples underling the need of a new regulation for these potentially toxic molecules.

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**EXTENDED AUTOMATION OF OLIVE OIL ANALYSIS  
ACCORDING TO CE REGULATION 2568/91**

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The commodity characterization of olive oils, mandatory at EU level, involves the evaluation of numerous parameters aimed at determining the class to which a given oil belongs.

Many of the required analyses involve a long sample preparation, characterized by the use of large volumes of solvents and consumables, as well as massive operator intervention.

SRA Instruments has developed a series of automations, validated and assessed in terms of robustness and reliability, based on the concept of green chemistry, aimed at determining Alkyl esters, Waxes, Sterols, Alcohols and Stigmastadienes. Such automations allow to:

- Drastically reduce the consumption of organic solvents. The volume of solvent required is about one tenth of that currently used
- Almost completely eliminate the use of consumables
- Hugely limit the operator's intervention
- Obtain a drastic reduction in analysis times
- Guarantee greater reliability of the analytical data.

These analytical platforms are based on HPLC/GC approach: once the sample is injected into HPLC, without any pre-treatment than dilution, the fraction containing the analytes of interest is withdrawn by the autosampler and stored in vial. Such fraction is, eventually, processed according to methods needs in a complete automatic way; once obtained the final extract, it is injected into a dedicated GC System.

All processes do not need any operator attendance, and analysis time is automatically optimized by controlling software.

**DETAILED ELUCIDATION OF INTACT LIPIDS IN FUNCTIONAL FOODS BY EXPLOITING A NOVEL IDENTIFICATION APPROACH BASED ON LINEAR RETENTION INDEX IN LIQUID CHROMATOGRAPHY – MASS SPECTROMETRY**

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The growing demand in natural matrices, representing a source of dietary and nutraceutical molecules, placed, as direct consequence, the urgent need for the development of suitable analytical methods able to provide a comprehensive characterization of both “conventional” and “unconventional” products.

In the last decades, lipidomics has emerged as a cutting-edge approach among omics- techniques, since lipids revealed to be essential molecules in the regulation of metabolic pathways. To this regard, the content of essential fatty acids (EFAs), as well as nutritional indices such as the levels of omega-3 and omega-6 FAs and their ratio are essential parameter to evaluate the beneficial properties of food products. In addition, the investigation of complex lipids in their native forms is proved to be crucial to obtain additional information about lipids role and on FA arrangement into each species.

For this reason, the present study is aimed to a detailed elucidation of intact lipids in different functional foods, including the profiling of microalgae, hemp products and the wastes of the fish industry.

Phospholipids and triacylglycerols were the most representative lipid classes. However, mono- and diacylglycerols, pigments and carotenoids were also detected, representing an added value for the investigated matrices.

From an analytical point of view, the use of a recently introduced linear retention index (LRI) approach in LC paved the way for the automatization of the identification process in LC. Furthermore, the use of high resolution chromatographic techniques (that is UHPLC), even combined with selective tandem MS operation mode allowed for the determination of the entire lipidome with high sensitivity. Finally, the UHPLC-MS/MS platform was coupled to a preparative workstation to fully automatize the analytical workflow

**COMBINED ANALYTICAL APPROACHES FOR CARBOHYDRATE CHARACTERIZATION OF LOW-FODMAP PRODUCTS AND NUTRACEUTICAL EXTRACTS FROM AGRO-INDUSTRIAL BY-PRODUCTS**

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In this project, a combined approach based on multiple analytical techniques has been exploited for carbohydrate characterization in complex matrices, such as foods and agro-industrial by-products. HPLC-SEC provides information on chain length distribution of different classes of sugars, including oligo- and polysaccharides; HPAEC-PAD is a high selective technique allowing separation and identification of each compound belonging to carbohydrates (alditols, amino sugars, mono-, oligo-, polysaccharides) according to their size; ATR-FTIR permits a rapid qualitative evaluation of a mixture of oligosaccharides. The information collected through these analytical tools offered the achievement of a complete characterization of the carbohydrates pattern in two different matrices: low-FODMAP (fermentable oligo-, di-, monosaccharides and polyols) products and agro-industrial byproducts.

FODMAPs are known for their prebiotic activity, but can rise several symptoms in patients affected by irritable bowel syndrome (IBS). HPAECPAD has also been used to study the effects of different ingredients and technological parameters during low-FODMAP bakery production. In particular, the selection of different chromatographic columns permitted a punctual separation of simple sugars and oligosaccharides.

Agro-industrial byproducts are source of fibers, such as fructo-oligosaccharides (FOS) and inulins, which can find interesting application as functional ingredients in the perspective of circular economy. The multiple analytical approach allowed the characterization of the different carbohydrates classes occurring in artichokes and onions by-products.

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**CONTROLLED PERIODIC ILLUMINATION AS VALUABLE STRATEGY FOR IMPROVING HER OVER METAL-SEMICONDUCTOR NANOPARTICLES AND STUDYING THE PHOTOCATALYTIC PROCESSES**

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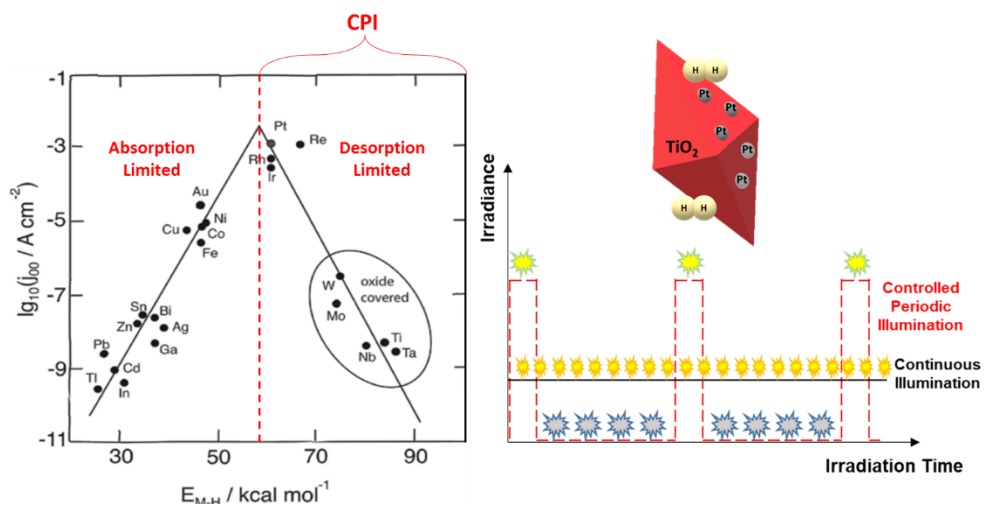
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In the next decades, the conversion of solar energy into electricity and solar fuels will be of crucial importance for a green and sustainable future. [1] The economic recovery after the pandemic will necessarily have to start by changing old paradigms with an energy transition towards renewable and clean sources. Italy has developed its own recovery plan (PNRR) in which 23.78 billion euros will be invested for the energy transition, of which 3.19 will be used to promote the production, distribution and use of hydrogen. [2] However, many challenges remain to produce and exploit it in an efficient way, in particular green hydrogen. [3]

In this context, water splitting using semiconductor photo-catalysts has been considered a sustainable method to produce clean hydrogen (H<sub>2</sub>) fuel. [4] Nevertheless, H<sub>2</sub> photo-production efficiency remains low, although extensive research effort has been carried out in these years about the mechanisms of the Hydrogen Evolution Reaction (HER) and the Oxygen Evolution Reaction (OER). [5] In this respect, TiO<sub>2</sub> is a key photoactive material, usually employed with a co-catalyst deposited onto the surface to enhance charge carriers' separation and catalyze surface charge transfer reactions. [6] Even with a noble metal catalyst, the hydrogen evolution reaction can be limited by the strong M-H bonding over some metals, such as Pt, Pd and Rh, inhibiting hydrogen desorption. H absorption is regulated by the potential at the metal nanoparticles. A possible strategy to enhance the hydrogen production could be the use of a Controlled Periodic Illumination (CPI). [8] Moreover, CPI can be a valuable tool to study HER mechanism. Assuming a fast variation of the photo-potential due to the alternation of light and dark periods, it is possible to favor the H<sub>2</sub> desorption from the metal surface. In this way it is possible to “climb” the Trasatti's volcano plot, increasing the efficiency of those catalysts that present a limitation in the desorption step. The hydrogen evolution rate and mechanism under CPI was studied with a combination of electrochemical, spectroscopic and chromatographic methods. CPI increased H<sub>2</sub> production rate in the presence

of Pt, Rh and Pd, whereas with Au and Cu no effect was observed. [9] These findings could also be useful for developing new sensor concepts.



**Figure 1.** Trasatti's Volcano Plot for the HER in acid solutions (graph from [7]) and schematic representation of Pt deposited nanoparticles on TiO<sub>2</sub> and CPI concept.

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## MICROPLASTIC IN DRINKING WATER: A NEW ANALYTICAL CHALLENGE

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Currently, *microplastic* pollution represents one of the most interesting environmental issues: to date, however, there is little data and knowledge about this type of contaminants.

Due to their heterogeneity and lack of standard classification criteria, there is no univocal definition of these pollutants: they represent a complex and heterogeneous class of compounds as they consist of polymers of different sizes (1  $\mu\text{m}$  -5 mm), types, shapes, and colors to which various additives and plasticizers are often added for technological reasons.

Thanks to the small size and chemical-physical properties, such as lightweight and resistance, microplastics are now widespread in the environment, where will remain for a long time, in fact, their high persistence makes it necessary to determine and characterize them. Microplastic pollution, indeed, is caused by the direct dispersion of these particles or by the degradation of plastic products or products that contain them, such as cosmetics [1] and toner.

Therefore, the prospect of being able to develop a monitoring strategy for these contaminants is important to reduce potential exposure and prevent any health effects.

Considering the complexity of this problem, a new challenge is represented by the development of an analytical method for their monitoring, in transposition of Directive (EU) 2020/2184 concerning the quality of water intended for human consumption [2].

The European Commission has set the aim of regulating the monitoring of these emerging contaminants by 2024, identifying a method for their determination, which allows the inclusion of the latter in the "Watch List".

Therefore, a first work is presented to fine-tune the method of identification and counting of microplastics using  $\mu$ -Raman spectroscopy for the qualitative-quantitative analysis of the latter in drinking water. Specifically, both the strengths and the critical issues of the method are highlighted, from

the sampling and sample pretreatment phase to the next step of analysis and processing of specific data, to develop an efficient method not only in terms of analysis but also of cost and effective operation in view of a future routine analysis.

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**BIOACCUMULATION AND ECOTOXICOLOGICAL EVALUATION OF BISPHENOL ANALOGUES IN *RUDITAPES PHILIPPINARUM*: FROM TARGETED ANALYSIS TO NON-TARGETED FINDINGS**

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Amongst emerging contaminants (ECs) exhibiting potential or well-known toxicological effects for humans and environment, endocrine disruptors (EDs) gained increasing attention because of their widespread use and consequent distribution in different environmental compartments and bioaccumulation along the trophic chain.

Bisphenol A (BPA) was extensively used as plasticizer in food and drink packages. Its use is nowadays restricted due to well documented negative effects on reproductive, immune, and neuroendocrine systems [1].

Bisphenol S (BPS), bisphenol F (BPF) and bisphenol AF (BPAF) are among the most studied BPA analogues. BPS, the most polar BPA analogue, is widely used in consumer products such as baby bottles or plastic containers, while BPF in coatings and epoxy resins and BPAF in electronic devices and optical fibers. As these chemicals are not covalently bound to the plastic polymers, they are easily released into the environment and evidence of their presence in various matrices have been reported in the last few years, in food, paper product, biological samples, and human tissues [2,3]. Furthermore, only few studies report bioaccumulation and ecotoxicological data regarding these molecules.

The present study aims to investigate the bioaccumulation of three BP analogues and evaluate their biochemical effects in the clam *Ruditapes philippinarum*, by combining LC-HMRS and biological assays. Animals were exposed to ppt levels of BPAF, BPF and BPS individually, as well as to a mix of the three, for 14 days. The level of BP analogues in clam tissues was measured by a targeted method, after optimization and validation of the extraction procedure. The variation of concentration of the BP analogues in the water of the tanks over a 24h period was also assessed. The biochemical response of clams toward the exposure to these contaminants was obtained by evaluating antioxidant biomarkers and hemocyte parameters.

As the animals were collected directly in the Lagoon of Venice before the acclimation in controlled tanks, and considering the power of HRMS, a non-

targeted analysis was also performed to evaluate the potential presence of other ECs already present in clams and water.

Preliminary results indicated that BPAF was bioaccumulated by clams (10-20 ng/g), while BPS and BPF bioaccumulation was limited in both tissues and organs. The different bioaccumulation rate of the targeted analytes was also coherent with the residual concentration in water obtained from the tank used to treat the animals. Finally, the non-targeted approach highlighted some other ECs occurring in clams collected in the Lagoon of Venice in view of the planned treatment with the BP analogues. The presence of these EC was also confirmed in the Lagoon water collected near the fishing area.

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## OPTIMIZATION OF A NEW MULTI-REAGENT PROCEDURE FOR QUANTITATIVE MUSSEL DIGESTION IN MICROPLASTIC ANALYSIS

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Microplastics (MPs) are considered one of the most serious environmental problems caused by anthropogenic activities. These micropollutants are widespread, particularly in the marine environment with several consequences for marine ecosystems and wildlife (1) also representing a potential threat to humans through seafood consumption (2). As a result, the determination of MPs in edible organisms is an important research topic and the scientific community is joining efforts to set up a general procedure fast, reliable, and cost-effective. The relevant analytical methods rely on extraction procedures of MPs from organisms by digestion. 10% KOH digestion demonstrated promising but affects MPs stability at longer treatment times. To reduce the digestion time and the risk of damaging MPs, a multi-reagent procedure, consisting of 2.5% KOH, methanol (0-20%) to enhance the proteolytic activity of KOH, H<sub>2</sub>O<sub>2</sub> (0-5%) to accelerate organic tissue degradation, in a temperature range between 60 and 90 °C for up to 3 h was exploited. Closed-vessel microwave-assisted digestion was used to reduce contamination risks, digestion time, and amount of chemicals. The design of experiments (DoE) enables to derive a statistical model to predict and optimize responses as a function of several factors (3) using a controlled number of experiments. A Full Factorial experimental design including the center points was used to identify experimental regions ensuring both complete tissue digestion and quantitative recoveries of MPs. Starting from the optimal conditions it was possible to find a robust set point: a mixture containing 2.5% KOH, 5% H<sub>2</sub>O<sub>2</sub> and 2.7% of CH<sub>3</sub>OH in 3 h at 60 °C gives a digestion efficiency greater than 98% along with the quantitative plastic recovery. The chemical stability of the tested polymers in the experimental conditions was confirmed by Raman spectroscopy. Finally, the optimized protocol was applied to identify MPs in mussels from local market and an average of 41 items/g dry weight was found.

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## OPTIMIZATION OF AN EASY, EFFECTIVE AND ROBUST PROTOCOL FOR THE EXTRACTION AND QUANTITATION OF MICROPLASTICS IN SOIL MATRICES

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The study of microplastic (MP) contamination in the environment is an emerging problem due to MPs ubiquitous presence, which causes both environmental and health issues. In this regard, the most investigated environmental compartment is the marine environment (e.g. oceans, sediments, etc.), in which it has been shown that MPs impact occurs both directly, through ingestion, and indirectly, since they convey other organic and inorganic pollutants.

Conversely, the presence of MPs in urban and agricultural soils is less investigated, despite the harmful effects they could also exploit in this compartment (e.g. reduction of fertility, alteration of biodiversity, transport of pollutants, etc.).

Based on these premises, in this work an analytical protocol for the extraction, quantification and recognition of MPs (PE-HD and PE-LD, PET, PA, PS both as particles and fibers) in urban and agricultural soils was developed. When treating the soil matrix, the presence of organic matter is the main obstacle since it interferes with the extraction of MPs. Hence, starting from a method previously developed in our laboratories for other environmental matrices, a first optimization step was performed, testing several oxidizing solutions (e.g. KOH, NaClO, H<sub>2</sub>O<sub>2</sub>, etc.) in their ability to remove organic matter, avoiding the degradation of polymers.

Subsequently, the procedure was further optimized, reducing the execution times, from about 96 to 72 hours per sample in respect to the original protocol. The extraction yields of the final optimized protocol (calculated on fortified samples) were satisfactory, with approximately quantitative values for all the polymers investigated, except for PET and PA (anyway higher than 80%). In addition, the method was shown not to alter the polymer lattice of plastics, making them recognizable at identification through FT-IR.

Finally, the developed method was successfully applied to the analysis of MPs in 4 soils of different physicochemical composition, evaluating also the effects of the chemical composition of the soil on the extraction yield of MPs, through a chemometric approach (protocol robustness).

**PROGRESS ON SOURCE ASSESSMENT OF POLLUTANTS REACHING THE POLAR REGIONS BY ELEMENTAL AND ISOTOPIC ANALYSIS**

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In many environmental studies, the determination of trace elements and their isotopic composition can provide valuable information on the geographical sources of both natural and anthropogenic inputs, the relative contributions of these sources over time and the corresponding transport routes. In fact, Pb shows pronounced variation in its isotopic composition because three out of its four isotopes, <sup>206</sup>Pb, <sup>207</sup>Pb and <sup>208</sup>Pb, are the stable end products of the decay chains of <sup>238</sup>U, <sup>235</sup>U and <sup>232</sup>Th, respectively. Therefore, there are relatively large differences in the isotopic ratios for crustal and ore Pb deriving from various locations. Contrary to Pb, Sr is typically of natural origin; however, since <sup>87</sup>Sr is produced via the  $\beta$ -decay of <sup>87</sup>Rb, natural variations of <sup>87</sup>Sr/<sup>86</sup>Sr in rocks occur, and the determination of this isotope ratio can be used for tracing the geographical sources of mineral dust. In addition, the elemental profile of a sample can help distinguish the various sources, mainly when data are processed by multivariate analysis techniques. The elemental and isotopic analysis of environmental samples collected from polar regions is, however, quite challenging, due to the low analytical concentration, matrix effects, risk of contamination and the limited sample size.

In the last decade, we have developed new analytical procedures based on inductively coupled plasma mass spectrometry, to accurately quantify trace elements and to measure the Pb and Sr isotopic ratios with adequate precision and accuracy. These methods were validated and successfully applied in the context of polar studies performed in both the Arctic (Ny-Ålesund, Svalbard Islands) and Antarctica (Terra Nova Bay and Dome Concordia).

In this communication, the latest analytical methods developed will be presented and discussed, along with the more recent results obtained in the context of the ongoing research projects in the Arctic and Antarctica.



**THE BURIED OPIUM: ANALYTICAL DETERMINATION OF ALKALOIDS IN ANCIENT REMAINS**

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The use of several psychoactive substances in antiquity is well assessed and related to its mystic significance, especially in rituals and in combination with traditional medicine and healing activities. The extraction of compounds from opium was recurring in ancient Egyptian civilization during the Bronze Age, as well as in ancient Greece. Opium use was also practiced by Roman population, where the first cases of dependence were evidenced [1]. In this framework, a peculiar case is represented by the population of *Dauni*, who inhabited *Apulia*, a region corresponding to the northern area of Puglia. In several vases from the VIII to VI century BC, belonging to the Ceci-Macrini collection, the depictions show references to poppy plants and use of opioids, probably in a religious context.

In this work, the development of an analytical method, involving sampling, extraction and LC-MS/MS analysis for the determination of opium alkaloids in archaeological complex matrices, is presented, in compliance with the principles of micro-invasiveness. Three sampling procedures were applied, based on previous methodologies used in archaeometry and forensics, while a versatile analyte extraction and clean-up method has been developed, consisting in Pressurized Liquid Extraction followed by dispersive Liquid-Liquid Micro-Extraction [2]. The extract was analyzed simultaneously in targeted and untargeted modes, to obtain a complete characterization of bioactive compounds eventually present in the investigated pottery. Thanks to the application of the presented method, several opium alkaloids were found (morphine, thebaine, quinine and codeine) in different ancient remains, while the untargeted analyses allowed isolating different natural compounds, such as roccellic acid, present in traditional infusions. These data would confirm the hypothesis about the use of opium poppy, and also of the plants of the Solenaceae and Chincona family, by *Dauni* in a ritual perspective [3].

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**DEVELOPMENT OF ANALYTICAL PROTOCOLS TO ASSESS THE PERFORMANCE OF NEW GREEN METHODS FOR RESTORATION**

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Nowadays there is an increasing demand related to the development of *ad hoc* materials for the restoration of cultural heritage. Several research activities are focused on the development of new green methods which are safe both towards the operator and of the environment. If on one hand the restorers are becoming extremely sensitive towards these issues, on the other hand they don't trust completely the new solutions and sometimes they are not prone to abandon traditional methods.

In this context, analytical protocols which are capable to compare the performances of the new green methods towards the traditional ones become extremely important to boost a change in the practice of the conservation community.

In the present research, we applied different analytical techniques to characterise and evaluate the performances of new green materials *ad hoc* developed for the selective removal of degraded or unwanted layers from different types of artistic artefacts (paintings on mobile supports, wall paintings, bronze statues, cinematographic movies) using traditional methods considered effective by restorers as benchmark.

The evaluation of the cleaning performances was performed considering the removal efficacy, the morphological alteration, and the retention of solvent.

## CHARACTERIZATION OF IN-LAB AGED LIPIDS BY USING GAS AND LIQUID CHROMATOGRAPHY APPROACHES COUPLED TO MASS SPECTROMETRY

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Lipids are the most widely studied compounds in archaeological pottery, thanks to their hydrophobicity that makes them particularly durable over time compared to other organic components [1]. However, most of the lipids are featured by reactive functional groups that fatally lead to their degradation in archaeological site, furtherly complicating the interpretations on the origin of lipid matter [2].

In-lab alteration processes such as thermal decomposition, oxidation and hydrolysis reactions have been already investigated [3]. Such ageing tests can provide key elements to study the formation of characteristic compounds and better address the origin of the organic archaeological remains. In the present study, in-lab thermo-oxidative treatments have been carried out to simulate the natural degradation of the lipid matter. In detail, standards of pure triglycerides such as triolein and trilinolein, and extra-virgin olive oil sample were kept in an oven at 120°C for three weeks. High and medium volatility components, including short-chain fatty acids, aldehydes, ketones, lactones and esters, were analyzed by using headspace solid-phase microextraction prior to gas chromatography-mass spectrometry (GC-MS), while low-volatility and polar compounds, such as medium- and long-chain fatty acids or ( $\alpha,\omega$ )-dicarboxylic acids, were converted into trimethylsilyl ethers (TMS) derivatives and analyzed by GC-MS. The degradation of the intact lipids was monitored by using non-aqueous reversed phase high performance liquid chromatography coupled to atmospheric pressure chemical ionization mass spectrometry.

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**STABLE ISOTOPE RATIO OF CARBON, NITROGEN, OXYGEN AND SULFUR: APPLICATIONS IN CULTURAL HERITAGE AND NEW ANALYTICAL METHODOLOGIES**

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Measurement of the ratios of naturally occurring stable isotopes (mostly hydrogen, carbon, nitrogen, oxygen and sulfur) is a useful tool in different fields of environmental science, food science, geochemistry, ecology, biology and also medicine. In fact, stable isotope ratio demonstrated the potential to differentiate between samples from different origin and source.

In the field of cultural heritage, we have recently applied stable isotope ratio to: (i) monitor air quality in historical monuments [1,2]; (ii) discover the origin of nitrate efflorescences on frescos [3]; (iii) identify the source of sulphur in black crusts formed on stone surfaces of monuments.

The preferred method for analysis of the stable isotope ratio at natural abundance is Isotope Ratio Mass Spectrometry (IRMS) thanks to its high accuracy and sensitivity. Due to the growing interest in this field, different analytical methodologies have been investigated for the measurement of carbon stable isotope ratio. Interestingly, we performed the measurement of  $\delta^{13}\text{C}$  values of inorganic carbonates, bicarbonates [4] and organic molecules such as vanillin [5] by using  $^{13}\text{C}$ -NMR spectroscopy without the need of IRMS.

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**POTENTIALLY HARMFUL ORGANIC COMPOUNDS LEACHED OUT FROM MICROPLASTICS: EXTRACTION METHODS AND DETECTION**

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Microplastics (MPs) have been detected in all environmental compartments, and the associated threats are the focus of intense scientific, and media attention. Synthetic polymers in the environment undergo degradation processes leading to mechanical and molecular fragmentation. We tested different extraction methods to evaluate the potentially harmful species leached out from MPs in the environment. Micronized reference polymers (LDPE, HDPE, PS, and PP) were artificially photoaged and investigated at different aging times. We applied evolved gas analysis-mass spectrometry (EGA-MS), analytical pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS), and size exclusion chromatography (SEC) to characterize the bulk polymers, their solvent extractable fractions, and the corresponding extraction residues. The analysis of the extracts and of the extraction residues allowed us to gain information on MPs degradation products and processes. Microwave assisted extraction combined with multi-shot Py-GC-MS enabled to detect the same degradation products in sand samples. The reference MPs were also subjected to accelerated photo-oxidative aging directly in artificial seawater, and the leached organic species were characterised and quantified by GC/MS. The analysis enabled to characterize different classes of compounds, such as linear and branched mono and dicarboxylic acids, and benzene derivatives. The results were compared with those obtained for the leachates from environmental plastic debris. Finally, solid phase extraction was tested directly on water leachates to evaluate the possibility to obtain complementary information compared to solvent extraction approaches. The combination of extraction methods and the analytical approaches proved effective in providing a better understanding of the chemical nature of degradation products released in the environment by different polymers in the micrometric size range. The research was part of the JPI Oceans project HOTMIC “Horizontal and vertical oceanic distribution, transport, and impact of microplastics (2020-2023)” and of the Fondazione Carilucca project “Micro and nano-plastics: quantification, evaluation of their impact on marine and lacustrine ecosystems, and remediation strategies (2020-2022)”.

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**CHEMICAL SPECIATION OF ATMOSPHERIC PM FROM POLAR REGIONS**

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The distribution of metals within the environment strongly depends on the formation of complexes with inorganic and organic ligands. These species can increase metal solubility, stabilize some of their oxidation states, activate some reactivity, and influence their bioavailability. In this work [1], the main ionic components ( $\text{Fe}^{2+/3+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{NH}_4^+$ ,  $\text{Cl}^-$ ,  $\text{NO}_3^-$ ,  $\text{PO}_4^{3-}$ ,  $\text{SO}_4^{2-}$ ,  $\text{CHOO}^-$ ,  $\text{CH}_3\text{COO}^-$ ) in the water-soluble fraction of Antarctic (Victoria Land) and Arctic (Svalbard Islands) PM have been quantified. A chemical model, based on the interaction between the components has been defined and applied to the samples to identify the species occurring in a water solution after the dissolution of the PM. The speciation studies showed that most cations were as aquoions over the whole pH range, except for Fe which occurred predominantly in hydrolytic form. To better simulate the conditions that can occur when the PM acts as cloud condensation nuclei, the concentration of the components has been increased up to 1000 times, keeping the ratio between them constant. The role of the inorganic anion as complexing agents became important only for an increment of the concentration of 100 times, and the  $\text{SO}_4^{2-}$  was the most involved in the formation of complexes. The organic complexing agents had minor importance, and a significant contribution has been observed only for those samples having a concentration of these components higher than  $10^{-5}$  mol L<sup>-1</sup>. Future studies will be necessary to improve the model using the formation constants and protonation constants of the ligands defined at lower temperatures (much similar to those of polar regions) and different ionic strengths. However, these first results allowed us to identify the chemical equilibria that most affect the soluble fraction of the metals bounded to the polar PM. This information contributes to a better knowledge of the atmospheric photochemistry processes and the biogeochemical cycles of these metals in the polar regions.



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**BERGAMOT PASTAZZO AS SUSTAINABLE PLATFORM FOR Cd<sup>2+</sup> REMOVAL FROM AQUEOUS SOLUTION**

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In the last years, citrus waste was employed as suitable platform for the development of multifunctional nanomaterials (MNMs), able to efficiently and selectively interact with organic (pesticides, dyes, drugs, POPs) and inorganic (metals, anions) contaminants, by means of sorption mechanisms [1, 2]. This contribution reports preliminary results on new biobased materials for Cd<sup>2+</sup> sequestration from multicomponent solutions simulating the conditions of natural fluids. The bergamot pastazzo was mechanically ground and divided in two fractions. A first aliquot (P1) was dried at  $t = 60^{\circ}\text{C}$  for 72 hours and pre-treated with various chemicals (*e.g.* NaOH, HNO<sub>3</sub>, H<sub>2</sub>O<sub>2</sub>, H<sub>2</sub>O, 2-propanol) to check for a possible enhancement of the pastazzo metal sorption capacity [3-5]. A second feedstock fraction (P2) was lyophilized, carbonized in an autoclave at  $t = 180^{\circ}\text{C}$  for 6 hours and purified in dialysis bags, to obtain carbon quantum dots (CQDs) [6]. P1 and P2 were characterized by FT-IR ATR spectroscopy to assess surface functional groups. Potentiometric studies were also performed at  $t = 25^{\circ}\text{C}$ ,  $I = 0.10 \text{ mol L}^{-1}$  in NaNO<sub>3(aq)</sub> to investigate their acid-base properties and binding ability towards Cd<sup>2+</sup>. Moreover, sorption capacity was investigated by means of kinetic and batch experiments, in which Cd<sup>2+</sup> concentration in solution was determined by DP-ASV and experimental data were analysed by various isotherm models [7].

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**PLASTICS IN THE MEDITERRANEAN SEA: INVESTIGATION OF THE PRESENCE IN TWO MARINE SPECIES AND POTENTIALLY CORRELATED ADVERSE EFFECTS**

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The ingestion of plastics by marine organisms is an issue well known which is raising concern worldwide.

Indeed, the accumulation of micro and macroplastics in the gastrointestinal tract can lead to physical damage, but also to chemical harmful effects related to the release of toxic plastic additives or waterborne persistent organic pollutants adsorbed onto the plastic particles [1].

In this work, the presence of macro and microplastics was evaluated in two species of the Mediterranean Sea: the Mediterranean small-spotted catshark (*Scyliorhinus canicula*, SC) and the Atlantic horse mackerel (*Trachurus trachurus*, TT) both collected during the Spring 2018 in two geographic locations in the southern region of the Mediterranean Sea (Lampedusa Island and near Mazara del Vallo).

The results showed that plastic ingestion is widely diffused in the two species from both locations, with microplastics (mainly polyester, polypropylene and polyamide) found in almost all the specimens analysed. Macroplastics were found with an average frequency of ingestion of 18% for SC both in Mazara del Vallo and Lampedusa, whereas for TT, macroplastics were found exclusively in a subset of larger specimens from Lampedusa.

Results from spleen and liver gene expression showed changes in the expression of three immune-related genes for SC specimens and anomalies in the production of vitellogenin in both males and females of TT.

Overall, our study suggests that the high frequencies of micro and macroplastics found in the two fish species considered could be a major cause for adverse effects in marine organisms, besides the interactions between the organisms and the wide variety of endocrine-disrupting substances present in seawater [1, 2].

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**DISINFECTION BY-PRODUCTS AND ECOTOXIC RISK ASSOCIATED WITH HYPOCHLORITE TREATMENT OF IRBESARTAN**

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Sartans are a class of chemical compounds with antagonistic action towards the AT1 receptors of angiotensin II, one of the main peptides responsible for regulating blood pressure. In just 20 years with their commercial success together with the low metabolic degradation and high environmental persistence sartans have been classified as emerging pollutants.[1,2] Nevertheless, the occurrence of pharmaceuticals in the environment should be reduced to minimize the risk of their distribution in any surface waters.[3] Irbesartan has been on the market for 19 years and has an annual production amount of approximately 20 tons. After its biological effect, irbesartan is mainly eliminated through the feces, although approximately 20% is found in the urine. The disinfection pathway of irbesartan was investigated by simulating the chlorination process normally used in a wastewater treatment plant (WWTP) to reduce similar emerging pollutants. The structures of ten new isolated disinfection by-products (DPs) have been determined by combining mass spectrometry and nuclear magnetic resonance data. A preliminary ecotoxicity assessment with the crustacean *Daphnia magna* showed that some of the identified byproducts were up to 12-times more toxic than irbesartan. The predicted toxicity is not "lower", but increased after chlorination due to chlorinated by-products. The discovery of these new molecules formed through the oxidation processes used in WWTP indicates their possible presence in the environment. The biological and abiotic pathways of these substances are unknown so, the side effects of the continuous exposure or adsorption through the food chain, should not be underestimated.

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**PHOTO-SENSITIZING PROPERTIES OF SUWANNEE RIVER NOM AND THEIR EVOLUTION UPON UVC LIGHT**

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In drinking water production Natural Organic Matter (NOM) is commonly considered an undesired constituent and is typically removed in the early stages of the treatment chain to avoid the formation of NOM toxic by-products. At the same time, in the environment NOM is the main responsible of the self-cleaning abilities of irradiated natural waters due to its ability to produce reactive species, upon absorption of solar light, capable of degrading organic contaminants. However, due to its complex and highly variable molecular structure, the correlations between NOM composition and its photo-sensitizing properties have not been fully elucidated yet.

In this study, Suwannee River NOM (SRNOM) was used to promote the degradation of organic water contaminants using two different strategies.

At first, the role of SRNOM as an iron complexing agent was investigated with the goal of performing a modified photo-Fenton process at circumneutral pH. The role of SRNOM concentration, iron concentration and oxidation state, and pH, on the removal of aqueous caffeine solution were evaluated under irradiation with simulated solar light ( $\lambda > 340$  nm). Increasing concentrations of SRNOM demonstrated to accelerate caffeine removal reasonably due to the formation of photo-active SRNOM-iron complexes. Caffeine degradation rate constant obtained in the optimized photo-Fenton carried out at pH 6 in the presence of SRNOM 40 mg/L was  $0.0183 \text{ min}^{-1}$ ; in comparison, a classical photo-Fenton carried out at pH 3 in the absence of NOM gave a rate constant of  $0.0126 \text{ min}^{-1}$ .

Secondly, the transformation of SRNOM irradiated at 254 nm and the evolution of its photo-sensitizing properties along irradiation were studied. It was observed that SRNOM that had been pre-irradiated for 3 hours at 254 nm could induce the photo-degradation of glyphosate 29 times faster than original SRNOM. Several SRNOM photo-active products were identified by means of high-resolution mass spectrometry and a reaction mechanism for the photochemical production of  $\text{HO}^\bullet$  was proposed.

The obtained results allowed to consider NOM presence in water as a possible useful resource to drive the degradation of organic contaminants present at low concentration, before the removal of NOM itself for drinking water production.

**UNTARGETED ANALYSIS OF ENVIRONMENTAL CONTAMINANTS IN SURFACE SNOW SAMPLES OF SVALBARD ISLANDS**

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In the recent years, there is an increasing attention on the contaminants of emerging concern (CECs), which include plasticizers, flame retardants, industrial chemicals, pharmaceutical and personal care products, since they have been revealed even far away from pollution sources. The Arctic is not exempt from the presence of anthropogenic contaminants, the increase of which is also connected with the increase of touristic and scientific activities [1], and the Norwegian Svalbard Archipelago is internationally adopted as a model for understanding the pollutant fate and impact [2].

During the 2021 spring campaign, sixteen surface snow samples were collected close to the town of Ny-Ålesund on the Spitsbergen Island of the Svalbard archipelago. The samples were subject to solid-phase extraction and analyzed by liquid chromatography-high-resolution tandem mass spectrometry following an untargeted approach. Compound tentative identification was obtained with the aid of the software Compound Discoverer, using both mass spectral database search and manual validation. Among the more than one hundred compounds identified with a high confidence level in all samples, about forty were anthropogenic contaminants (mainly drugs and plasticizers). Nonetheless, a clear contamination trend did not appear in the snow samples collected on different days; therefore, particulate matter collected on filters in the same area was analyzed too. The detection of only a few compounds and mainly of natural origin in common between snow and filter samples suggested that, in this case, the atmospheric transport was not relevant for CEC presence in the sampling site.

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**CRITICAL EVALUATION OF CHALLENGES AND LIMITATIONS  
IN POLYURETHANE MICROPLASTICS ANALYSIS BY PY-GC/MS**

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Polyurethanes (PUR) consist of a broad and heterogenous group of polymers, widespread in every aspect of our everyday life. The large market demand, and the variety of application fields justify the inclusion of PUR in microplastic (MP) investigation. PUR analysis by pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS) is challenging due to the lack of a unique thermal degradation product that allows the analysis of the whole class at once. This study aimed at providing comprehensive information on PUR in the analysis of MPs by Py-GC/MS to clarify whether (i) it is possible to make a reliable statement on the PURs content of environmental samples based on a few, characteristic thermal decomposition products and (ii) which restrictions are required in this context. Methylene diphenyl diisocyanate and toluene diisocyanate PUR (MDI-PUR and TDI-PUR, respectively) were pyrolyzed directly, and under thermohemolytic conditions (addition of tetramethylammonium hydroxide, TMAH). Pyrolytic indicators were identified and thermochemolysis was defined the preferred analytical method even though it entailed a loss in sensitivity and incomplete methylation for sterically hindered amine groups. Regressions in the 1-20 µg mass range showed good correlation ( $R^2 = 0.91 - 0.96$ ). ANOVA tests on respective slopes underlined that quantitation behaviour of different MDI-PURs can be represented by the calibration of one representative MDI-PUR with sufficient accuracy, entailing a good estimation of the entire subclass despite of varying structures (p-value=0.982). However, precision and sensitivities resulted affected by particle size and mass ranges. As a suitability test of the method road dusts and spider webs were collected around a plastic processing plant and compared with data of a previous study [1]. Dried samples were milled, sieved, oxidised by Fenton's reagent and filtered. Pyrograms were analysed for MPs including the selected PUR indicators. Procedural blanks enabled consideration of any laboratory contamination. The detection of MDI-PUR was highly reliant by the proximity to a potential source. TDI markers were not revealed. TMAH-Py-GC/MS allowed a good estimation of MPs PUR content, under some limitations discussed above.

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**DETECTION OF SIDEROPHORE-TYPE LIGANDS IN SEA WATER SAMPLES VIA SOLID PHASE EXTRACTION AND HPLC-ESI-MS/MS**

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Speciation of dissolved iron (dFe) in the oceans has been shown to be dominated by complexation with strong organic ligands [1]. Several studies suggest that siderophores are likely to make up some component of the organic ligands that control dFe speciation [2][3][4]. Siderophores have low molecular masses and are produced by microorganisms to sequester and assimilate dFe, under low Fe conditions. Siderophores coordinated to insoluble Fe(III) are first transported into microbial cells by membrane bound Fe-siderophore receptors. Afterwards, Fe is then released from siderophores, typical via reduction of Fe(III) to Fe(II) by microbe-mediated redox processes [2][5]. Siderophores are a structurally diverse group, that can be classified by their metal binding groups as catecholates, hydroxamates, carboxylate or in a heterogenous group with other metal-binding ligands called complexones [6][3]. The study of these ligands is crucial to better understand the biogeochemical cycle of Fe in the marine environment especially in some oceanic areas, such as the Southern Ocean, where Fe deficiency limits marine productivity. The characterization of these compounds is very difficult, due to their complex structures and low concentrations in seawater. Large sample volumes and specific techniques for the extraction and pre-concentration of these compounds are generally needed. In this study, an analytical method is proposed for the extraction and pre-concentration of siderophores from seawater samples by Solid Phase Extraction (SPE) followed by High performance liquid chromatography - electrospray ionization - mass spectrometry (HPLC-ESI-MS/MS) analysis. A central composite design was used to optimize recovery of extraction from seawater made through SPE C18 cartridges using MeOH as eluent. Ferrioxamine E, a commercially available hydroxamate siderophore, and Staphylopherrin A, a siderophore with two citric acid units obtained by solid-phase synthesis, were used as standard. The HPLC-ESI-MS/MS procedure was adapted from the method already developed by the research group for the identification of extracellular polymeric substances in sea ice [7]. A C18 reversed phase column was used for the chromatographic separation with a mobile phase composed of A) 0.1% (v/v) formic acid in water and B) 0.05% (v/v) formic acid in acetonitrile. The procedure was then applied to the analysis of samples collected in the Ross Sea (Antarctica) during the austral summer 2020.



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**AN INTEGRATED ANALYTICAL APPROACH TO INVESTIGATE THE INTERACTION BETWEEN MICROPLASTICS AND (TRACE) ELEMENTS IN ENVIRONMENTAL SAMPLES**

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The interaction of plastic particles with (trace) elements recently attracted the attention of researchers investigating its possible environmental implications. Plastic ageing and water chemistry seems to regulate this interaction, but the mechanisms are still unclear [1]. Accordingly, the analysis of plastic environmental samples plays a crucial role in understanding different species adsorbed on plastic surface, possibly shedding light on the interaction mechanisms [2]. Still, this type of analysis represents a challenging analytical task: elemental concentration can be at trace level, sample size is limited, the matrix is extremely inhomogeneous, and samples are difficult to handle.

Therefore, in this study we propose an integrated analytical approach to test the most likely chemical species adsorbed on microplastic, including leaching extractions and direct instrumental methods. Different single and sequential extractions were tested (to analyse physisorbed, carbonate-bonded and organic matter-bonded metals) and acid digestion of samples (to obtain bulk concentration), followed by inductively coupled plasma – mass spectrometry. Surface characterization of functional groups (using infrared spectroscopy) and micromorphology (using scanning electron microscopy) were then integrated to evaluate the likely bonding sites for (trace) elements.

The speciation results indicated a significant difference between environmental and pristine samples. The former generally enrich in different micronutrients (Fe, Zn, Sr and Mn) and other trace elements (Sn and Ba), especially in the more extractable phases (physisorbed and acid soluble phases). Moreover, this enrichment in (trace) elements well correlates with infrared bands typical of plastic ageing and biofouling (e.g., amides and polysaccharides), highlighting a pivotal role of these processes in governing plastic-element interaction.

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## CONVERSION OF RICE HUSK BIOMASS INTO SORPTIVE CARBON NANOMATERIALS FOR STEROID HORMONES IN ENVIRONMENTAL WATERS

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Rice husk (RH) is a non-food waste biomass with high silica content (15–28%) that does not allow its composting and use as animal feed [1]. Its insolubility in water and the presence of carboxylic and silanol functional groups in the organic components (ca. 72–85 wt%, including lignin, cellulose, and hemicellulose) make RH an excellent starting material for many organic and inorganic compounds [1, 2].

In this work RH char obtained from pyrolysis (700°C, 2 h, N<sub>2</sub> flow) was oxidated by H<sub>2</sub>SO<sub>4</sub> and HNO<sub>3</sub> evaluating different times and char amount/solvent volume ratio. After an oven treatment in a high-pressure Teflon vessel (200° C, 10 h) the obtained products were investigated through TEM analysis observing nanoparticle aggregates (20–30 nm). The preparation procedure was also expanded to an industrial air-burned RH to evaluate a possible large-scale reuse of this agro-food waste gaining similar results in terms of nanomaterial morphology.

Solid-Phase Extraction (SPE) tests were carried out with the aim to evaluate both adsorption and elution. Preliminary trials were performed on 25 mg of each material in tap water samples (50 mL, native pH) enriched with 2 µg/L of 16 steroid hormones (8 Glucocorticoids, 3 Estrogens, 2 Progestins and 2 Androgens) as probe xenobiotics representative of water emerging contaminants. All compounds were quantitatively adsorbed from water evidencing a good affinity for the materials. The elution was performed with 2 mL of acidic methanol and the eluates were analyzed by HPLC-ESI-MS/MS (Multiple Reaction Monitoring mode), achieving quantitative recoveries for all the analytes with both materials (> 80%, RSD% < 15, *n* = 3).

Driving by these results, further tests were performed with larger volumes of environmental water samples increasing the amount of sorbent (50 mg). In details, SPE tests were carried out in river and lake water samples (100 mL) enriched with 2 µg/L of the 16 compounds obtaining high recoveries (> 80%, *n* = 2) for all the analytes except Progesterone (~ 60%).

Preconcentration tests are ongoing in actual water samples (river waters and wastewater treatment plants effluents) at lower concentrations (ng/L).

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## MICROPLASTICS: POTENTIAL IMPACTS ON THE OCEAN'S BIOGEOCHEMISTRY

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One of the major challenges in understanding the dynamics of the ocean's functioning is the potential impact of the increasing presence of plastics, both macro and micro. Besides impacts on marine wildlife and habitats, micro and macroplastics offer potential sites for microbial activity and chemical leaching. Most marine plastic is found initially in the upper meters of the water column, where fundamental biogeochemical processes drive marine productivity and food web dynamics. However, recent findings show a continuum of potential effects on the fundamental dynamics of the carbon and nutrient cycles(1).

Microplastics can act as substrates for microbial activity and can influence biomass production. This has potentially important implications at the sea-surface microlayer, the marine boundary layer that controls gas exchange with the atmosphere and where biologically produced compounds can accumulate. In large scale mesocosm experiments and controlled laboratory experiments, we explored variations in the dynamics of naturally produced dissolved organic matter, particulate organic matter and marine gels in the sea-surface microlayer, in the presence and absence of microplastic contamination of the underlying water. We show that microplastics increased biomass production and enrichment of particulate carbohydrates and proteins in the sea-surface microlayer. The increase has both direct and indirect impacts on gas exchange between the marine surface waters and the atmosphere, with important implications CO<sub>2</sub> exchange.

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**SAMPLING AND MOLECULAR DETECTION OF BIOAEROSOLS:  
AIRBORNE BACTERIA IN A WASTE WATER TREATMENT  
PLANT AND SARS-COV-2 IN A POORLY AERATED ROOM WITH  
A MILD SYMPTOMATIC SUBJECT**

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Bacterial and viral bioaerosol sampling and detection for outdoor and indoor environmental samples do not have standardized norms, despite the relevance of impacts of airborne pathogens, as witnessed by the COVID-19 pandemic and diffusion of AntiMicrobial Resistance in healthcare environments. We report an intercomparison of sampling approaches based on Condensation Growth Tubes (Aerosol Devices-ViVAS sampler), turbulence impingers (SKC-Biosamplers), gelatine impactors and filters (Sartorius-MD8 Airport). Detection is based on rt-PCR also with metagenomic or metabarcoding approach for bacteria on 16S gene [Mbareche, 2017], and targeted detection of SARS-CoV-2 by CANARY detection [Rider, 2003]. Methodological pros and cons will be presented, as well as case studies on bacteria from aeration tanks of untreated effluents from a Waste Water Treatment Plant and details from repeated measures by ViVAS, CANARY, swab samples and residual infectivity as tested in a BSL3 laboratory by plaque test on VERO E6, from a isolation room being poorly aerated (controlled by CO2 monitoring). Elective applicability and weaknesses of different procedures will be highlighted, contributing to provide basis for sound bioaerosol characterization.

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**NON-TARGET SCREENING WITH HIGH RESOLUTION MASS SPECTROMETRY FOR THE IDENTIFICATION OF EMERGING CONTAMINANTS IN SURFACE WATERS AND WASTEWATER EFFLUENTS**

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Chemical contaminants in aquatic ecosystems have been an emerging concern, and our knowledge of the chemicals present is still limited. Non-target screening (NTS) based on high-resolution mass spectrometry (HRMS) has immense potential to help in overcoming these limitations.

Here we used NTS analysis to screen contaminants of emerging concern (CECs) in the aquatic environment. The study was conducted as part of the H2020-MSCA-ITN-2017 project AQUALity (Project No. 765860). In two campaigns, a total of 34 samples were collected from rivers, lakes, and industrial and municipal WWTP effluents in France, Greece, and Italy. Samples were extracted using solid phase extraction (SPE) with Oasis HLB (200 mg) and ENVI-Carb Plus (400 mg) cartridges. Each sampling site had three SPE-extracted blanks of ultrapure water. Samples were analyzed using an LC-HRMS system (Orbitrap or Q-TOF) and the acquired data were processed using vendor software, followed by MZmine 2 or MS-DIAL.

Overall, 673 compounds were identified following the scheme proposed by Schymanski et al. [1] with HRMS databases such as ChemSpider and MassBank. The identified compounds were predominantly pharmaceuticals and personal care products (PPCPs) and pesticides, followed by food additives, human metabolites, and transformation products. Furthermore, 28 compounds were commonly found in both sampling campaigns, potentially indicating their spread in the environment. Information regarding the 673 identified compounds and LC-HRMS data have been deposited into the NORMAN database.

This study demonstrated the potential of NTS based on HRMS in identifying CECs in aqueous systems. However, further research is needed to identify more of the unknown compounds that were detected. Finally, quantification and toxicological studies are required to evaluate the relevance and risk of the and newly identified compounds.

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**VERSATILE FLUORESCENT PIGMENTS FROM AN ANTARCTIC BACTERIUM: CHARACTERIZATION AND ENVIRONMENTAL APPLICATION**

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Siderophores are important secondary microbial metabolites that chelate iron and, in recent years, the study of their application in the environmental remediation of metals has been intensified, due to the strong affinity, towards metals other than iron [1]. These research, may in the future, provide a viable, economically, and ecologically advantageous alternative to the more dangerous and invasive remediation methods involving the use of synthetic chemicals.

Pyoverdine is a siderophore, characterized by a specific fluorescent dihydroxyquinoline chromophore, and different dicarboxylic-side and peptide chain, bounded to the amino group and to the carboxyl group of the chromophore, respectively, that are specific for each bacterial species [2]. More than 100 pyoverdines have been identified from different bacteria; although these are structurally different, they nevertheless share common features [3]. Until now, few studies have been characterized these pigments and elucidate their peculiar properties.

In this study, a mix of Pyoverdines (PYs), produced by *Marinomonas sp. Efl*, a bacterium isolated from a consortium associated with the Antarctic ciliate *Euplotes Focardii*, was purified, and chemically analysed. The biosynthesized PYs are pale yellow, water-soluble and fluorescent. After purification, spectrophotometric and fluorometric characterization were carried out. HPLC analysis performed by using DAD, FLD and MS detectors, demonstrated that the sample was composed by a mix of seven pyoverdines and the relative masses were obtained. The complexing ability for different metal ions was also investigated by spectrophotometric and fluorometric measurements. The obtained results showed that different metal ions are complexed from PYs, while Cr(VI), that in the studied pH conditions was present as  $\text{CrO}_4^{2-}$ , is reduced to Cr(III) and successively complexed. This important result offers the possibility to use this pyoverdines mix in the control of Cr(VI) pollution.

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**FAST LC-MS/MS SCREENING METHOD FOR THE EVALUATION OF DRUGS, ILLICIT DRUGS, AND OTHER COMPOUNDS IN UNCONVENTIONAL BIOLOGICAL MATRICES**

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In the last decade, the consumption of illicit substances, the widespread diffusion of the NPSs, and the low presence of new device for *non-invasive* sampling continue to cause concern. One of the major limitations still present today is related to the availability of validated confirmation methods. The analytical challenge involves the variety and complexity of the biological matrices to be investigated, with particular attention to unconventional matrices, which have gained great interest in recent years for their drug detection window, less invasive sample collection and reduced adulteration. Moreover, the greatest challenge is also related to the development of fast and robust methods, with minimal sample manipulation to avoid target analytes loss. In this work a rapid screening procedure in liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS) for the evaluation of 739 compounds in unconventional matrices (blood, post-mortem blood, urine, EBA) has been reported [1,2]. This method represents a powerful tool for the monitoring of high number of analytes thanks to the instrumental configuration and to the protocol of acquisition of the instrumental signal.

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**A NOVEL AZA-PATERNÒ-BÜCHI REACTION FOR PINPOINTING CARBON-CARBON DOUBLE BONDS IN LIPIDS BY HIGHER COLLISIONAL DISSOCIATION MASS SPECTROMETRY**

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The evaluation of double bond positions in fatty acyl chains has always been of great concern since relevant studies have pointed out their significance in the chemical and biochemical role of lipids [1]. Despite being the foremost technique for lipidomics, it is difficult in practice to obtain identification beyond the fatty acyl level by the sole high-resolution mass spectrometry. As such, the location of C=C bonds cannot be effectively achieved by tandem MS experiments with the common collision-induced dissociation (CID) techniques [2]. In the latest years, several innovative approaches for pinpointing carbon-carbon double bonds have been proposed, such as ozone-induced dissociation inside the mass spectrometer and chemical derivatization before analysis. Paternò-Büchi (PB) reactions of fatty acids with ketones have been successfully proposed for pinpointing double bonds in fatty acids, as PB-derivatized lipids generate diagnostic product ions under CID fragmentation [3]. Despite the large body of literature on PB reactions and their variants, higher collisional dissociation (HCD) has never been used in combination with PB reactions.

In the present study, we propose for the first time a methodology based on the aza-Paternò-Büchi (aPB) reaction of lipids with 6-azauracil and HCD for pinpointing fatty acid double bonds. Similar to PB reactions, aPB reactions are [2+2] photocycloadditions between excited imine and alkene groups that generate four-membered azetidines [4]. The introduction of the 6-azauracil moiety allowed enhancing the ionization efficiency of the fatty acid ions both in positive and negative ion mode due to the presence of ionizable sites for both polarities. The method was optimized using free fatty acid and phospholipid analytical standards and compared to the standard PB reaction with acetone. As a result, the 6-azauracil moiety allowed obtaining protonated fatty acid ions and deprotonated phosphatidylcholine ions. Moreover, the aPB reaction furnished higher ionization efficiencies in both polarities and clearer HCD spectra in negative ion mode compared to the PB reaction, which in turn was found barely suitable with HCD. The method was finally applied for pinpointing C=C bonds in 64 polar lipids from a yeast extract.

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**AUTOMATIC AND MINIATURIZED EXTRACTION COUPLED WITH ON LINE UHPLC-MS/MS FOR THE ANALYSIS OF VITAMIN D METABOLITES AND INTACT LIPIDS FROM DRIED BLOOD SPOT**

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Lipidomics is the metabolomics branch that studies lipids within a living system. Lipids are involved in many vital biological processes, and their dysregulation contributes to several negative human conditions, such as cardiovascular disease, diabetes, obesity, cystic fibrosis, male and female infertility, and Alzheimer's disease. Recently, the lipidomics approach opened new insight toward the determination of the native lipid composition. In fact, monitoring intact lipids can reflect more deeply the regulation of lipid metabolism in response to exogenous stimuli and provide elucidations on the perturbation of essential metabolic processes in which each species is involved. Among the different lipid classes that can be identified and quantified in whole blood, fat-soluble vitamins, and especially Vitamin D, are of particular interest. Vitamin D plays a crucial role in phospho-calcic metabolism, and in recent years many other important protective effects have been highlighted. Its action is due to several metabolites that are activated through various steps, and render its determination a challenging task; mainly due to the choice of the metabolite to be evaluated and the broad range of their concentrations in the blood. Ultra-high performance liquid chromatography coupled to mass spectrometry (UHPLC-MS) represents the technique of choice for the study of intact lipids. In this work, LC-MS/MS was used to investigate the presence of Vitamin D metabolites and the total lipid composition of whole blood spotted on Dried Blood Spot. The instrument was coupled to a fully automated preparative station, able to perform the extraction of the lipid fraction from the samples prior to the injection in the instrument, increasing the reproducibility of the analysis and minimizing the errors due to the handling of the samples by the operators.

**PROGRAMMABLE BIOSENSING INTERFACES BASED ON DYNAMIC DNA SYSTEMS INTEGRATED WITH NANOSTRUCTURED ELECTRODES**

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DNA nanotechnologies can be engineered into molecular transduction systems and integrated with a variety of materials interfaces to design specific nucleic acid biosensors. Carbon nanotube (CNT) electrodes are cheap, highly conductive, and robust, and are therefore ideal substrates for the fabrication of electrochemical sensors. Controlling functional DNA systems on a CNT surface, however, is a challenging task requiring both a fine tuning of hybridization processes at the materials interface and strategies to limit non-specific physisorption contributing to false positives. Here, we provide design rules for new classes of biosensors that integrate dynamic DNA systems with single-walled carbon nanotube electrodes and show that specific DNA interactions can be monitored and measured by using redox-tagged DNA strands that yield an amperometric signal. We demonstrate the use of pyrene as a backfilling agent to reduce non-specific adsorption of DNA strands and report the controlled formation of DNA duplexes on the electrode surface. This supported the development of novel amperometric hybridization platforms based on programmable DNA strand displacement reactions and on artificial DNA structures templated by the small molecule melamine, which allowed for performing strand exchange reactions that can prove useful in the design of new bioelectrochemistry approaches.<sup>[1]</sup> Substituting a linear DNA probe with a DNA aptamer sequence is a strategy for engineering the same electrode interface into a sensing platform for the recognition of a specific protein based on affinity binding. Following the same functionalization protocols, a redox-tagged DNA aptamer that specifically binds to the receptor binding domain of the SARS-CoV-2 spike protein S1 subunit was immobilized onto the same CNT electrodes, enabling the single-step, reagent-free detection in the nanomolar range of the S1 protein through a binding-induced folding mechanism that results in a controlled suppression of the amperometric signal provided by the electroactive tag.<sup>[2]</sup>

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**RABBIT IgG-IMPRINTED NANOMIPS BY SOLID PHASE SYNTHESIS AS MIMIC OF PROTEIN A: EFFECT OF CROSS-LINKER ON AFFINITY AND SELECTIVITY**

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Immunoglobulins G (IgG) are extremely relevant in analytical applications where very high selectivity towards a target molecule is mandatory, as affinity chromatography, (bio)sensoristics and immunoassays. Affinity chromatography based on Protein A, a glycoprotein with high affinity for the Fc region of IgG, is the preferred method for high-throughput purification of IgG. However, this method suffers from high costs and limited stability of Protein A, and harsh elution conditions which can sometimes lead to irreversibly damage IgG. Thus, man-made IgG-binding materials based on the molecular imprinting technology which could overcome these drawbacks are of significant interest. The solid phase synthesis (SPS) of molecularly imprinted nanoparticles (nanoMIPs) represents an innovative method to prepare nanomaterials with tailor-made molecular recognition properties towards peptides and protein. The synthesis of nanoMIPs by SPS usually involves a pre-polymerization formulation where the cross-linker is invariably N,N'-methylene-bis-acrylamide (BIS). To date, the effect on the binding properties of nanoMIPs using other than BIS cross-linkers has never been reported. In this work, in order to investigate the effect of different cross-linkers in protein-imprinted nanoMIPs prepared by SPS, alongside BIS we considered other similar cross-linkers: N,N'-ethylene dimethacrylamide (EDAM), N,O-bis-methacryloyl ethanolamine (NOBE), ethylene glycol dimethacrylate (EDMA) and glycerol dimethacrylate (GDMA), replacing them for the BIS in pre-polymerization mixtures. Synthesized nanoMIPs were homogeneous, with a polydispersity index of 0.24-0.30 and mean diameter of 129-169 nm in water. The binding properties of the nanoMIPs were measured by equilibrium partition experiments with the template, rabbit IgG (RIgG), and selectivity was evaluated with respect to bovine IgG (BIgG), bovine serum albumin (BSA) and hen egg lysozyme (LZM). The experimental results show that all the cross-linkers, with the exception of EDMA, gave nanoMIPs with high binding affinities for the template (BIS:  $16.0 \times 10^6 \text{ mol}^{-1} \text{ L}$ , EDAM:  $8.8 \times 10^6 \text{ mol}^{-1} \text{ L}$ , NOBE:  $15.8 \times 10^6 \text{ mol}^{-1} \text{ L}$ , GDMA  $12.8 \times 10^6 \text{ mol}^{-1} \text{ L}$ ), medium to high imprinting factors (BIS: 12.3, EDAM: 5.5, NOBE: 7.2, GDMA 11.6) and selectivity towards other proteins comparable to Protein A but markedly dependent on the structure of the cross-linker, confirming the importance of the latter in the SPS of imprinted nanoparticles.

**FAST DETERMINATION OF ENDOCANNABINOIDS AND RELATED COMPOUNDS BY MEANS OF UHPLC-MS/MS IN BIOLOGICAL MATRICES**

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Endocannabinoid system (ECS) was widely studied due to its involvement on several pathologic conditions and its contribution on homeostasis balance by different ways. ECS explicates its action through specific neurotransmitters called endocannabinoid (eCBs) [1]. Different compounds, structurally related to the major eCBs, were often identified as "cannabimimetic", due to their capability to bind fully or partially CB1 and CB2, the main ECS receptors, or to inhibit the eCBs degradation process. In this scenario new ECS related compounds, called N-arachidonoyl conjugated eCBs, raised as important neurotransmitters and modulators of classic eCBs levels, which derives from the conjugation between arachidonic acid and other bioactive molecules [2]. Their low concentration in biological matrices makes them a challenging analytical task [3]. Usually, eCBs extraction requires large amount of sample for analysis by means liquid/solid extraction (LLE) or solid phase extraction (SPE).

The aim of this work was the development of a fast and reliable analytical method to determinate principal eCBs and related compounds, especially N-acylaminoacids. To this aim a  $\mu$ SPE-LC-MS/MS method was developed: miniaturized SPE led to reduced sample amount, maintaining high sensibility of the method, and it was able to detect principal eCBs, secondary eCBs, 5 N-acylaminoacids and dopamine conjugated compound. The presented method provided also very low matrix effect and good extraction recoveries both in plasma and brain samples, which are the most used matrices in in vivo studies, for their biological significance.

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**ANALYTICAL WORKFLOW FOR SULFOPEPTIDE ENRICHMENT AND SEQUENCE ANALYSIS WITH SITE LOCALIZATION**

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Protein tyrosine O-sulfation is an important post-translational modification correlated to inflammation, virus infection, and signal pathways. Analytical methods for enrichment and detailed study of sulfopeptides, able to provide peptide sequence and site localization, are currently limited due to issues with low abundance and poor stability of the sulfate modification[1]. In this context, an enrichment method was developed for two commercial peptides, representative of mono- and di-sulfated peptides, by comparing five sorbent materials, i.e. two commercial weak anion exchange mixed mode sorbents three phosphopeptide enrichment materials[2]. Recoveries were studied by UHPLC-multiple reaction monitoring analysis. The Fe-IMAC kit provided recoveries >80% from spiked bovine serum albumin digests and good selectivity. The enrichment was tested on serum samples within a shotgun proteomics workflow with a protein dephosphorylation step and tryptic digestion. The recovery of the entire analytical workflow was 20%, which was compatible with previous data on TiO<sub>2</sub> phosphopeptide enrichment. The sulfate detection and localization issue was studied by different fragmentation techniques (CID, HCD, ETD, EThcD, ETciD) and compared to the related phosphorylated counterpart sequences. All tested conditions were suitable for phosphopeptide analysis, but for intact sulfopeptides only the sequence could be obtained. However, the use of metal adduct precursors, especially potassium ones, improved the stability of the sulfate modification and provided information on both sequence and site localization of sulfate under ETD and ETD-hybrid strategies. In-source neutral loss of SO<sub>3</sub> and under EThcD provided diagnostic peaks suitable to distinguish the sulfopeptides from the nearly isobaric phosphopeptides.

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**UNTARGETED LIPIDOMICS TO EVALUATE THE EFFECTS OF X-RAY IRRADIATION ON MOZZARELLA CHEESE**

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X-ray irradiation is an emerging non-thermal technology which allows to preserve the hygienic quality of food and extend its shelf-life, by inactivating and destroying pathogenic and/or deteriorating microorganisms [1-2]. In this study, irradiation treatment was applied to mozzarella cheese and untargeted lipidomic approach was used in order to characterize the lipid profiles of not-irradiated and irradiated samples at 1.0, 2.0 and 3.0 kGy, with consequent evaluation of any safety issue regarding lipid alteration. The lipid extraction procedure was based on a slightly modified Folch method and the extracts were analyzed by means of UHPLC-Q-Orbitrap-MS. Finally, LipidSearch<sup>TM</sup> software was used for reliable and accurate molecular identification. A total of 382 lipids, categorised into 13 subclasses, including ceramides (Cer), diacylglycerols (DG), triacylglycerols (TG), phosphatidylcholines (PC), phosphatidylethanolamines (PEt), phosphatidylinositols (PI), phosphatidylserines (PS), sphingomyelins (SM), bismethyl phosphatidic acids (BisMePA), cholesterol ester (ChE), hexosyl ceramides (HexCer), monogalactosyl diacylglycerol (MGDG) and digalactosyl diacylglycerol (DGDG) were measured. Furthermore, to better understand the lipid oxidation mechanisms in irradiated mozzarella cheese, the identification of possible oxidized lipids was also taken into consideration setting dedicated search parameters in LipidSearch<sup>TM</sup>. Chemometric approach was able to highlight differences among irradiated and non-irradiated samples. The results confirmed that this “omic” approach is a powerful tool to provide useful information on lipid changes induced by X-ray irradiation.

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## PHYTOCANNABINOMICS: A TOOL FOR CANNABIS CHEMOVAR DIFFERENTIATION AND COMPOUND DISCOVERY

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*Cannabis sativa* L. is a flowering annual plant with the ability to produce a characteristic class of terpenophenolic compounds called phytocannabinoids. Among the over 150 members, the best-known are  $\Delta^9$ -tetrahydrocannabinol ( $\Delta^9$ -THC), cannabidiol (CBD) and cannabigerol (CBG), along with their carboxylated precursors  $\Delta^9$ -tetrahydrocannabinolic acid ( $\Delta^9$ -THCA), cannabidiolic acid (CBDA) and cannabigerolic acid (CBGA). Indeed, the chemotype distinction of cannabis varieties is classically based on the relative abundance of these compounds. However, this approach is limiting due to the complexity of the pool of phytocannabinoids present in each variety. *Phytocannabinomics* aims at a comprehensive characterization of the phytocannabinoid composition through an untargeted metabolomics approach. To this end, liquid chromatography coupled to high-resolution Orbitrap mass spectrometry (HPLC-HRMS) and a customized database were used for the putative identification of over a hundred phytocannabinoids [1]. Then, chemometrics analysis allowed to identify several new subgroups within the traditional chemotype classifications arising from a unique composition of the minor phytocannabinoids [2]. Among these less abundant compounds, the presence of the *cis* isomer of the carboxylated form of  $\Delta^9$ -THC, *cis*- $\Delta^9$ -tetrahydrocannabinolic acid (*cis*- $\Delta^9$ -THCA), emerged for the first time in the plant material. *Cis*- $\Delta^9$ -THCA was characterized and quantified in several cannabis samples from different chemotypes by HPLC-HRMS, along with the other main species (CBGA, CBG, *trans*- $\Delta^9$ -THCA, *trans*- $\Delta^9$ -THC, CBDA, CBD, cannabichromenic acid (CBCA), and cannabichromene (CBC)). This approach proved to be useful not only for the discovery of new phytocannabinoids but also for the evaluation of potential relationships between phytocannabinome and pharmacological effects.

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**MASS SPECTROMETRY-BASED ASSAY FOR THE DETERMINATION OF NATRIURETIC PEPTIDES IN PLASMA SAMPLES**

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Cardiac natriuretic peptides (NPs), i.e., ANP, BNP, and CNP, are hormones initially synthesized from cardiomyocytes, as prohormones. Once they are released from the heart, prohormones are split into two fragments: a longer N-terminal fragment (NT-proBNP and NT-proANP) and a shorter C-terminal fragment (BNP and ANP) [1]. In pathological conditions as heart failure (HF), heart induces the stretching of myocytes due to pressure overload, leading to the release of NPs in blood. Thus, NPs plasma levels increase progressively with the severity of HF and represent the main biomarkers for prognosis and diagnosis of HF [2]. NPs are normally determined in blood using commercial immunoassay kits [3]. This approach is affected by cross-reactions due to the presence of several NP forms showing homologous structures [3], as those with oxidized methionine [4]. In this work we developed a reliable and innovative analytical workflow based on targeted top-down proteomics and UHPLC-MS/MS analysis for the determination of NPs in saliva and plasma samples. Peptides' ionization efficiency was improved by the addition of a supercharger agent into mobile phases, which allowed to enhance up to 10-times the sensitivity of the method, depending on the analyte. In the optimized conditions, the instrumental detection limits were in the range 10-1000 pg/mL. Particular attention was paid to the evaluation of analyte stability in standard solutions as well as in biological specimens. NPs were found to be stabilized at 4°C in aqueous solutions by the presence of both human serum albumin, as displacement agent, and methionine as oxygen scavenger up to 24h. Ultrafiltration, fractional protein precipitation, and micro-extraction by packed sorbent (MEPS) were tested as sample treatment procedures. The optimized analytical protocol was employed for the monitoring of patients suffering from HF.

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**MALDI IMAGING-BASED SPATIAL-OMICS HIGHLIGHTS SPECIFIC METABOLITE AND LIPID SIGNATURES OF PAROTID TUMOR**

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Salivary gland tumors are relatively uncommon neoplasms that represent less than 5% of head and neck neoplasms, and about 90% are in the parotid gland [1]. Due to the wide variety of histologies and the heterogeneous tumor characteristics, their diagnosis can be challenging, hence novel approaches are needed to improve their treatment. In the present study, MALDI Mass Spectrometry Imaging (MSI) has been employed to discriminate the pathological regions of patient-derived biopsies of parotid neoplasms, as well as to inspect the spatial modulation of metabolism in tumor tissues. Metabolite and lipid profiles were simultaneously mapped in consecutive sections of 22 fresh-frozen human parotid tissue samples by MALDI-TOF MSI analysis, both in positive and negative ionization mode, at a lateral resolution of 50  $\mu\text{m}$ . In addition, MALDI-FT-ICR MSI experiments were performed, for accurate metabolite annotation. MALDI-TOF MSI datasets were subjected to spatial segmentation and compared with the histologic annotation. In order to maximize the information related to specific alterations between the pathological and healthy tissues, unsupervised (PCA) and supervised (PLS-DA) multivariate analyses were performed, presenting a 95.00 % accuracy in cross-validation. Characteristic lipids and metabolites signatures were highlighted for tumor and healthy adjacent parotid regions. In particular, glycerophosphocholines and glycerophosphoethanolamines were significantly increased in tumor regions, while sphingomyelins and triacylglycerols, which are key players in the signaling pathway and energy production of neoplastic cells, were all sensibly reduced. In addition, a significant increase of amino acids and the purine/pyrimidine metabolites in tumor, consistently with the bioenergetics request of cells, was observed. These results underline the potential of MALDI-MSI as a complementary diagnostic tool to highlight the altered metabolic pathways in salivary gland tumor and provide potential biomarkers to improve the specificity of diagnosis and treatment.

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**SYNTHESIS OF Fe<sub>3</sub>O<sub>4</sub>-ACTIVATED CARBON FROM WASTEPAPER HANDKERCHIEFS FOR DISPERSIVE MAGNETIC SOLID-PHASE EXTRACTION OF NON-STEROIDAL ANTI-INFLAMMATORY DRUGS NSAIDS AND THEIR UHPLC-PDA DETERMINATION IN HUMAN PLASMA**

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Sample preparation is one of the most important and critical steps of a chemical analysis. It greatly affects the quality of the data and the analysis time in biological fluids. The techniques mainly used for the extraction of NSAIDs are protein precipitation, liquid-liquid extraction (LLE), and solid phase extraction (SPE). In recent years carbon-based materials such as MWCNTS/Fe<sub>3</sub>O<sub>4</sub>, GO-Fe<sub>3</sub>O<sub>4</sub>, rG-Fe<sub>3</sub>O<sub>4</sub> and activated carbon- Fe<sub>3</sub>O<sub>4</sub> have been widely used as sorbents for the extraction and enrichment of analytes from various matrices. As well as carbon-based materials, metal-organic frameworks have attracted the attention of the scientific community due to their easy synthesis, high porosity, and large surface area, which make them the ideal sorbents in magnetic solid-phase extraction (MSPE).

However, the high production cost of metal organic-frameworks limits their diffusion as sorbent for the MSPE. For this reason, in this work we prepare activated carbon-Fe<sub>3</sub>O<sub>4</sub> with a carbothermal reduction method by combining ferric chloride and wastepaper handkerchiefs. The wastepaper handkerchiefs were used as carbon source for generating Fe<sub>3</sub>O<sub>4</sub>, giving a residual porous carbon which has high adsorption capacity. The obtained sorbent was characterized by Scanning Electron Microscopy (SEM), thermogravimetric analysis (TGA), Energy Dispersive X-ray analysis (EDX), X-Ray Diffraction analysis (XRD) and Raman spectroscopy. Subsequently, the sorbent was applied in the magnetic-solid phase extraction of NSAID from human plasma. The MSPE-UHPLC-PDA method was validated, and the parameters influencing the extraction were evaluated in order to maximize the recovery of the analytes. The method has been successfully applied in the magnetic solid phase extraction of NSAIDs from human plasma with excellent results. Furthermore, the produced sorbent has allowed it to be reused up to ten times while maintaining constant results. This method can lead the way in using cellulose-based high value-added resources.

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**USE OF ONLINE BUFFER EXCHANGE COUPLED TO NATIVE MASS SPECTROMETRY TO ELUCIDATE SUBSTRATE-BINDING AFFINITY OF FrlB, A BACTERIAL DEGLYCASE**

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Amadori compounds are stable sugar-amino acid conjugates that are formed non-enzymatically via the Maillard reaction. Fructose-lysine (F-Lys,  $\epsilon$ -conjugated) is one of the most abundant Amadori compounds in processed foods and is a key intermediate in the formation of advanced glycation end products, which in turn are implicated in inflammation and disease. FrlB, a deglycase, converts 6-phospho-fructose-lysine (6-P-F-Lys) to L-lysine and glucose 6-phosphate, with the latter feeding into glycolysis among other routes.<sup>1</sup>

Native Mass Spectrometry (nMS) is a powerful analytical tool that allows the study of macromolecular complexes in the gas phase where proteins remain folded and non-covalent interactions can be preserved. The general workflow is based on protein buffer exchange into a solution of a volatile salt (usually ammonium acetate) followed by nano-ESI-MS analysis. Online buffer exchange coupled to nMS (OBE-nMS) is an approach that does not require extra sample preparation. With OBE, samples were kept in a non-volatile buffer that favors their native biological properties and then buffer exchanged into ammonium acetate on-line for nMS analysis.<sup>2-5</sup> In the present study, OBE-nMS was used to confirm the oligomeric state of FrlB and to investigate 6-P-F-Lys binding affinity.

Our OBE-nMS studies show that FrlB exists as a dimer and, when treated with 6-P-F-Lys, can bind 6-P-F-Lys and retain two copies of the substrate per dimer (at a protein:ligand ratio 1:400). These results agree with kinetic studies conducted on FrlB in the presence of the substrate. The protein-ligand binding results obtained by OBE-nMS are also comparable with the protein-ligand binding data collected by using nanospray-MS, confirming the robustness of the OBE-nMS method for protein-ligand screening.

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## A NUTRIMETABOLOMICS APPROACH TO UNDERSTAND PROTECTION FROM SARS-COV-2 INFECTION

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Although the recent mass vaccination campaign has opened a new chapter in the battle against SARS-CoV-2, COVID-19 pandemic is still raging in most countries. The application of metabolomics in the nutrition sciences has been used not only to discover new food intake biomarkers but also to assess dietary compliance or dietary patterns. Foods and food ingredients play an important role in achieving or maintaining a state of wellbeing. The potential contributions of foods and bioactive substances to prevent COVID-19 have not been explored yet.

In the current study, we used nutrimetabolomics to identify chemicals and variables associated with a high risk of developing COVID-19 infection<sup>1</sup>.

Untargeted metabolomics analysis was performed on a first cohort of 50 serum samples collected from healthy healthcare professionals at the onset of the COVID-19 pandemic in Italy and who were later exposed to the same risk for contracting COVID-19. Of these subjects, half developed COVID-19 within three weeks of the blood collection. Small molecules were extracted from serum, derivatized and then analyzed using bi-dimensional gas chromatography/mass spectrometer (GCxGC-MS). The metabolomics profiling between the two groups were then compared.

We identified the presence of a metabolic phenotype associated with protection from SARS-CoV-2 infection and predisposition to COVID-19. Among molecules correlated to protection we identified several fatty acids and amino acids that could be used as dietary supplements. In particular, we identified an important role of monolaurin, which has well-known antiviral and antibacterial properties. The molecule was higher in protected subjects, suggesting a potential defensive role against SARS-CoV-2 infection; thus, dietary supplements could boost the immune system against this infection. A recent trial showed that virgin coconut oil, which is mainly composed of monolaurin, was able to reduce inflammation in COVID-19 patients.

We then quantified monolaurin and lauric acid on a larger cohort of subjects composed by 800 healthcare workers. We then correlated the levels of these molecules with the time to infection with SARS-CoV-2. The validation phase confirmed the potential protective role of monolaurin and its derivative, especially in male subjects.



This study shows that metabolomics can be of great help for developing personalized medicine and for supporting public healthcare strategies.

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**NATIVE FFF-CHARACTERIZATION AND QC PROFILING OF HUMAN AMNIOTIC STEM CELL VESICULAR FRACTIONS FOR SECRETOME-BASED THERAPY**

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Human amniotic mesenchymal stromal cells (hAMSCs) have unique immunomodulatory properties making them attractive candidates for regenerative applications in inflammatory diseases. Most of their beneficial properties are mediated through their secretome, showing paracrine effects. The precise nature of the bioactive factors concurring to its therapeutic activity are still unknown, but evidence points to the synergy between its molecular and vesicular fractions in regulating the acute/chronic inflammation shift and ultimately stimulating self-healing and functional regeneration. Vesicular secretome contains a multitude of particles with varying size, cargos and functions whose complexity hinders full characterization and comprehension. Moreover, biological variability of the starting samples, deriving from placenta treatments, and the lack of a specific QC protocol, are relevant obstacles for the translation to clinical applications of secretome-based therapies in humans. In this work a crucial step forward was obtained by exploiting native FFF-based separation and characterization of hMSC derived secretome: the informations obtained allow to estimate protein content, evaluate particle size, distribution and obtain native fractions. This approach is effective both as a stand-alone profiling approach to determine quality, helping to shift towards method standardization, and as tool to simplify and unscramble populations prior to LC-MS and ELISA, which allowed to categorize unique and recurrent proteins and clusters related to vesicle activity and functions. .

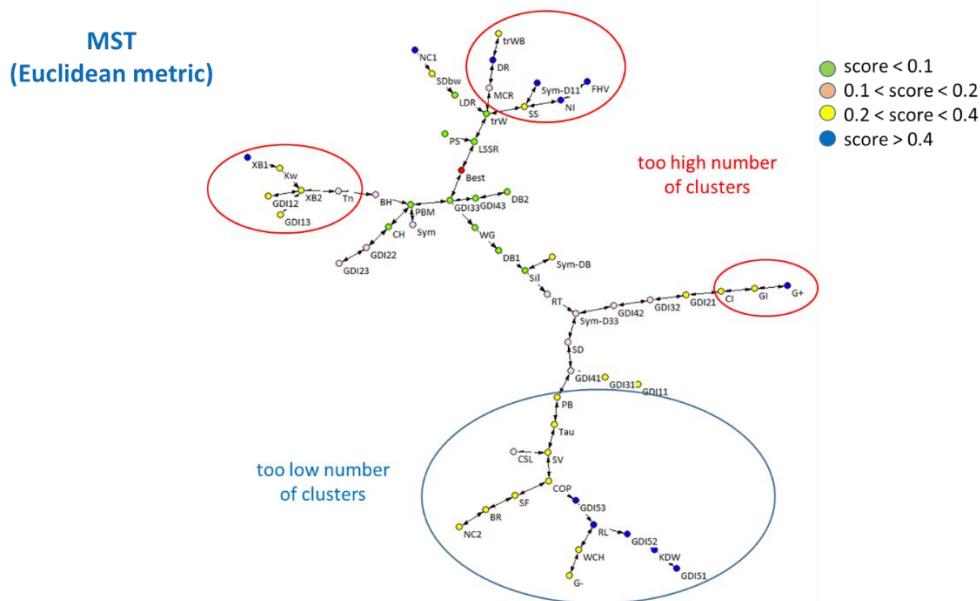
**THE CLUSTER VALIDITY INDICES: A COMPREHENSIVE REVIEW AND MULTIPLE COMPARISON BY CHEMOMETRICS METHODS.**

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*Cluster validity indices* are mathematical functions aimed to detect the optimal number of clusters in which a data set can be possibly partitioned. In this work, 61 indices proposed in the literature since 1965 were analyzed on 11 real and 10 simulated data sets.

The obtained results were compared with the targets by different scoring functions and chemometrics methods. In particular, Minimum Spanning Tree and Principal Component Analysis allowed highlighting a subset of reliable indices able to detect a number of clusters always equal to or near the target. A Minimum Spanning Tree example is shown in the figure below.



**PROSTATE CANCER DETECTION BY EXCITATION-EMISSION  
FLUORESCENCE SPECTROSCOPY OF URINE COUPLED WITH  
CHEMOMETRICS**

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In the present study, excitation-emission Fluorescence Spectroscopy, has been investigated as a rapid and accurate analytical method for the early screening of prostate cancer directly through urine analysis in order to provide reliable results while improving patient compliance.

Previous studies with fluorescence spectroscopy [1] have shown optimal results in the investigation of cancer patients of different etiology, but this work, to the best of author's knowledge, represents the first attempt in the discrimination of prostate cancer patients to normal ones through the application of advanced multivariate data strategies to elaborate EEMs spectra.

69 urine samples (46 samples of patients with malignant prostate cancer and 23 samples of healthy donors) were analyzed. Urine samples were taken after pathological diagnosis of prostate cancer and before any kind of medical and/or surgical treatment. Urine samples were taken with a sterile procedure and immediately frozen at -80°C until analysis. The EEM fluorescence measurements were performed on centrifugated urine samples at room temperature on a Perkin-Elmer LS55B luminescence spectrometer. The excitation spectra were recorded between 250 nm and 530 nm each 5 nm (29 recorded points), whereas the emission wavelengths ranged from 270 nm to 650 nm each 0.5 nm (761 points).

From a synergistic analysis of the obtained results, four fluorescent bands, corresponding to four selected PARAFAC components, were recognizable in the urine EEMs and the respective species seemed to be potential markers in the differentiation among healthy and cancer samples. PARAFAC results, in terms of extracted scores, coupled with PLS-DA algorithm, allowed to develop a first attempt of healthy/cancer discrimination model. The relatively low number of samples could be a limitation for the robustness of the obtained results; however, the obtained results could surely contribute to the development of a simple and non-invasive protocol for prostate cancer detection to be used as a screening tool able to support the different techniques used in this issue.

## CHEMO-OR02

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**NEW STRATEGIES FOR MULTI-BLOCK DATA ANALYSIS BASED ON THE ROSA ALGORITHM**

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Recently, response-oriented sequential alternation (ROSA) was presented as a novel method for multiblock data analysis [1]. With respect to competitive approaches for multi-block data analysis, ROSA exploits a “winner takes all” approach, where each component is calculated from the block of predictors that leads to the minimum residual error, to produce accurate model in exceptionally rapid computational times.

Starting from the original formulation, which was meant for the prediction of a univariate response, we developed a multi-response version which was then extended to cope also with discriminant classification problems. In the present communication, these extensions of the original ROSA algorithm will be discussed together with their application to real and simulated data sets.

Moreover, some solutions for addressing the most relevant drawback of the ROSA algorithm, i.e., the fact that the choice of the successive components can be heavily affected but the previously extracted ones, will also be presented.

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**UNTARGETED METABOLOMIC APPROACHES IN FOOD IDENTIFICATION: THE CASE OF PARMIGIANO REGGIANO PDO CHEESE CERTIFICATION “PRODOTTO DI MONTAGNA PROGETTO TERRITORIO”**

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The potential of metabolomics as a robust and efficient analytical tool in food authentication is widely recognized [1-2]. In this study, two different analytical tools, uHPLC-MS and NMR, were used as fingerprinting (untargeted) strategies in a context of *food identification*, i.e. to define the identity of Parmigiano Reggiano “Prodotto di Montagna Progetto Territorio, PdM, [3] based on the characteristic features which make it unique. In particular, the case of Parmigiano Reggiano PDO certification “Prodotto di Montagna Progetto Territorio” was investigated since this high added value product plays an important role in supporting the sustainability of the mountain areas where it is produced, offering revenue opportunities for local economy. In this preliminary study, 40 cheese samples, 20 PdM and 20 Parmigiano Reggiano PDO (hereafter indicated as *conventional PDO*), provided by *Consorzio del formaggio Parmigiano Reggiano*, were analysed by UHPLC/HRMS and NMR profiles, with an untargeted approach in order to characterize the compositional profile of the Parmigiano Reggiano “Prodotto di Montagna Progetto Territorio” samples as well as to find putative markers able to differentiate them from *conventional PDO* ones. Multivariate Curve Resolution methodology, such as ROI-MCR [4], and interval MCR, coupled to other chemometrics tools allowed resolution of overlapped signals and depicting ‘identification’ features. The resolved features were putatively identified by different reference spectral libraries (i.e. Chemomx NMR Suite, and those present in Compound Discoverer). Finally, Parmigiano Reggiano “Prodotto di Montagna Progetto Territorio” samples resulted well differentiated with respect to *conventional PDO* samples, in terms of amino acids and oligopeptides content, which are the markers showing the highest discrimination potential.

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**DEVELOPMENT OF ULTRASENSITIVE IMMUNOMETRIC BIOELECTRONIC SENSORS WITH EXPERIMENTAL DESIGN**

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The US National Institute of Health defines biomarkers as molecules that can be objectively measured and evaluated as indicators of normal or disease processes and pharmacologic responses to therapeutic intervention. Among the plethora of biomarkers, the sensitive detection of proteins is of paramount importance in a number of clinical fields.<sup>1</sup> The clinical use of protein biomarkers as indicators of the onset of pathological states requires the measurement of low concentrations of proteins in complex samples. Attempts to develop ultra-sensitive assays for the detection of protein biomarkers have been done by several groups in the last few years. Although in the last decade many approaches to achieve ultra-sensitive detection have been developed, most of them require complicated assay set-ups, hindering their adoption in point-of-care applications. In this perspective, Electrolyte-Gated Field-Effect-Transistors (EG-FETs)<sup>2-6</sup> with a bio-functionalized gate electrode, appear as very promising biosensing platforms. The EG-FET device herein presented, able to operate in physiologically relevant fluids such as blood serum and saliva, will set the ground to a major revolution in biosensing applications for early clinical detection. An experimental design approach has been undertaken leading to the optimization of the sensing protocol, improving the biosensor analytical figures of merit and reducing the cost of the assay.

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**DESIGN OF EXPERIMENTS AND RESPONSE SURFACE METHODOLOGY AS POWERFUL TOOLS FOR THE OPTIMIZATION OF SAMPLE PREPARATION AND INSTRUMENTAL ANALYSIS**

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The Design of Experiments (DoE) is a multivariate approach aimed at a rational plan of the experiments, which allows to compute mathematical models able to describe our system. It permits to understand how the variables influence a response and interact among each other, avoiding the costly and long “one variable at a time” strategy [1]. Depending on the purpose, it is possible to choose among a selection of experimental designs, such as Plackett-Burman, Box-Behnken and central composite designs. In our lab, DoE has been successfully implemented to both sample treatment and instrumental analysis, in different applications, herein presented.

If several variables are involved in complex problems, a first screening followed by a response surface design may be necessary. This was the case of the optimization of the analysis of five phytoestrogens in soy-food. A Plackett-Burman design allowed to select the significant variables affecting the accuracy; then, Box-Behnken design was employed to build the response surfaces and find the optimal experimental conditions.

In the analysis of 21 bioactive compounds by non-aqueous reverse phase chromatography, a simple central composite design allowed to see how flow, temperature and gradient velocity influenced chromatographic separation. Just seventeen runs (less than one day of analyses) were necessary to gather the data and then define the best compromise to separate coeluted peaks.

The optimization of a dispersive solid phase extraction for polycyclic aromatic hydrocarbons (PAHs) in seawater was rapidly achieved thanks to the Plackett-Burman design. This screening design (just 12 experiments) allowed to reveal that only one variable out of 9 significantly affected the recovery, thus avoiding the performance of a large number of experiments. In a similar application to botanical supplements, the D-optimal design was employed for optimization. The knowledge acquired in the previous study permitted to select only 4 variables: their effect on the PAHs recovery was studied by performing 18 experiments. The response surfaces allowed to rapidly identify the significant variables and optimal conditions.

In all these case studies DoE demonstrated to be a powerful tool to obtain the maximum information with the minimum experimental effort.

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**DEVELOPMENT OF A METHOD FOR THE IDENTIFICATION OF DEFECTS IN STEEL COILS BY SEM, IMAGE ANALYSIS AND MULTIVARIATE STATISTICS**

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Stainless steel is usually produced in laminates rolled in coils which can be further marketed in standard or made-to-measure formats. The quality control of these products is carried out both through chemical investigations and through the evaluation of defects present on the surface of the laminates: the presence of defects of different types makes the product unusable for some of the intended purposes. The superficial investigation of the defects is carried out through a scanning electron microscope (SEM) and the evaluation of the defects present is done manually by the operator, with an evident waste of time.

The aim of the project is therefore to evaluate the possibility of using image analysis methods to perform an automatic analysis of the images produced through the SEM technology in order to automatically identify defective samples from exempt samples. The defects that may be present are of different types: presence of knotty oxides, inclusions, rust and various combinations of the previous defects.

The images produced during the industrial quality control were then collected, performing magnifications at 50X, 100X and 200X. The images were processed using ImageJ in order to highlight the defects with respect to the background. The product of this processing is an image composed of black pixels, representing the structures of the defects, and white pixels, representing the background. The black and white images were then further processed through an algorithm developed ad hoc to calculate different morphological indices of the defects to describe their characteristics (elongation, number of structures, repetitiveness, etc.). The resulting dataset was subjected to multivariate statistical analysis for the determination of a classification model, through PLS-DA (Partial Least Squares - Discriminant Analysis) coupled with variable selection methods and by an approach based on Kohonen supervised self-associated neural networks (Supervised SOMs). The performance of the models obtained is very good, even in cross-validation, making the use of specific descriptors and the calculation of the PLS-DA model a possible method for the automatic classification of defects with respect to controls. The application of Artificial Neural Networks has also proved to be a promising strategy for the classification of controls and morphological defects.

**MULTIVARIATE IMAGE ANALYSIS FOR IN-FIELD  
QUANTIFICATION OF ANTHOCYANINS CONTENT**

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The use of analytical techniques for fast and non-destructive determination of food chemical composition is rapidly increasing, thanks to the possibility of extracting the information of interest by proper chemometric elaboration of signals/images that can be acquired using advanced technologies available at low costs. In this context, we have recently developed a device for the determination of the anthocyanins content of red grapes directly in the vineyard, based on the elaboration of image data acquired using a smartphone camera. The device essentially consists of a hardware part (3D-printed controlled lighting acquisition camera equipped with LED lights, colour reference, grape sample and smartphone housings) and a software interface (smartphone app coupled to a web interface), allowing the winery to monitor in real time the anthocyanin values measured in the different areas of the vineyard and their evolution with time. The core of the device is a chemometric software that allows to determine the parameters of interest, i.e., total and extractable anthocyanins, starting from the RGB image of the grape sample. This presentation is mainly focused on the description of the multivariate image analysis strategy developed to obtain robust calibration models, which essentially consists of three steps: i) image standardization to account for possible variations of the lighting conditions; ii) conversion of the image data into signals, namely colourgrams [1], which codify the distribution of colour-related parameters within the images; iii) development and external validation of PLS regression models that use colourgrams to estimate the parameters of interest. The performance of the device was tested in the frame of the Emilia Romagna Region PSR project “VITEVEN” in collaboration with the main regional wine producers, considering three different wine varieties (Lambrusco Salamino, Ancellotta and Sangiovese) located in nine different vineyards during the 2020 and 2021 harvest years. Satisfactory results were obtained in particular for the prediction of the parameters of interest for Lambrusco Salamino and Sangiovese, with  $R^2_{\text{PRED}}$  values ranging from 0.71 to 0.85.

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**FUTURE FOODS: AUTHENTICATION OF CRICKET AND BUFFALO WORM FLOURS FOR HUMAN CONSUMPTION**

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In recent years, the possibility of reintroducing edible insects as “future foods” in the human diet has been reevaluated also in areas where this practice is not traditional. The reasons of this lie in the noticeable nutritional properties of some edible insects (generally rich in protein, vitamins, mineral elements, polyphenols and fatty acids) and their sustainable breeding which requires a moderate amount of water, and no peculiar exigencies. Consequently, insect flours and insect-based snacks are currently spreading on the market all over the world. Given the recent interest in these aliments, it is still necessary to work on their characterization and authentication. The present work aims at investigating the characteristics of cricket and buffalo worm flours and at developing an infrared spectroscopy (IR)-based tool for detecting adulterations in insects' flours for human consumption. Mixtures of cricket and buffalo worm flours, prepared to mimic adulterated samples, together with pure flours of the same insects, have been analysed by FT-IR spectroscopy. Eventually, classification tools for discriminating between pure and adulterated samples, i.e., Sequential Preprocessing through ORThogonalization Discriminant Analysis (SPORT-DA) and Soft Independent Modelling of Class Analogy (SIMCA), have been employed. Both discriminant and class-modelling approaches demonstrated to be suitable for the purpose. The highest correct classification rate on cricket flour was achieved by means of SPORT-DA, which correctly predicted 100% of test samples; whereas, the most accurate predictions on the buffalo worm flour data set were obtained using SIMCA, which allowed the erroneous classification of only three (over thirty) test objects.

## FIRST-ROW TRANSITION METAL CATIONS COMPLEXES OF LUTEOLIN: STABILITY CONSTANTS AND STRUCTURAL CHARACTERIZATION IN AQUEOUS MEDIA

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Over the last decades, an increasing interest has been shown towards flavonoids [1], mainly due to their potent antioxidant activity, which is essential in preventing and treating oxidation damage. Besides being able to act as primary antioxidants, donating an H atom to stabilize a radical species, flavonoids can act as secondary antioxidants due to their ability to chelate pro-oxidant metal ions. This second antioxidant mechanism may result from the interactions between metal ions and flavonoids to produce complexes. Among the various classes of flavonoids, flavones are characterized by a 2-phenyl-1,4-benzopyrone skeleton and are usually classified by the number and position of their hydroxy and methoxy substituents. Luteolin (3',4',5,7-tetrahydroxyflavone, H<sub>4</sub>Lu, Fig. 1) occurs in the leaves, blossoms, and stems of various plants and has been recently used in many areas of medicine, food chemistry and biochemistry.

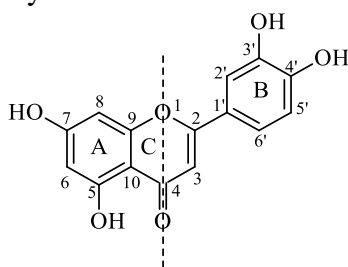


Figure 1. Chemical structure of luteolin, H<sub>4</sub>Lu

Luteolin possesses two possible chelating sites: the 5-hydroxy-4-oxo and 3',4'-dihydroxyl (catechol) groups. The chelation of metal ions can be crucial in preventing radical generation, which can cause damage to biomolecules. Following our previous studies on the sequestering ability of luteolin towards Al(III), Fe(III) and Cu(II) [2], herein we present an experimental investigation on the complexation ability of luteolin towards first-row transition metal cations at 37 °C and in 0.16 M NaClO<sub>4</sub>, evaluating the stability constants of the complexes and the corresponding structures.

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## ON THE ACID-BASE AND METAL IONS ADSORPTIVE PROPERTIES OF COLLOIDAL CARBON-BASED NANOPARTICLES

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Graphene related materials have been of great interest in nanotechnologies as promising materials of many technological applications due to their remarkable electrical, mechanical, thermal and reactivity properties<sup>1,2</sup>. Most applications of carbon-based materials harness their ability to interact with specific atoms, ions, and molecules through chemical surface groups. In this work, acid-base and metal ions adsorptive properties of hydrophilic carbonaceous nanoparticles (HNPs), with uniform dimensions and a good grade of dispersion in water, were investigated and modeled. HNPs, bearing a variable number of oxygen-containing functional groups at the surface, were obtained through the oxidation of a carbon black with nitric acid, at a temperature of 100  C, for different reaction times (2- 24 hours).<sup>3</sup> The acid-base properties of the functional groups were evaluated in water suspension by potentiometry and infrared spectroscopy. By means of X-ray fluorescence, infrared spectroscopy and differential pulse voltammetry the interaction properties of HNPs with Cd<sup>2+</sup>, Zn<sup>2+</sup>, Pb<sup>2+</sup>, Ln<sup>3+</sup> ions have also been investigated.

The experimental data, collected in the 2.0 to 7.0 pH range, suggest that the adsorption of metal ions on HNPs is controlled by chemical adsorption involving the strong complexation of metal ions with the carboxylic groups on the surface of HNPs.<sup>4</sup> Particularly, the surface high complexation of Ln<sup>3+</sup> ions open the exploitation of HNPs in solid phase extraction for preconcentration, separation and determination of Rare Earth Elements and Actinides.

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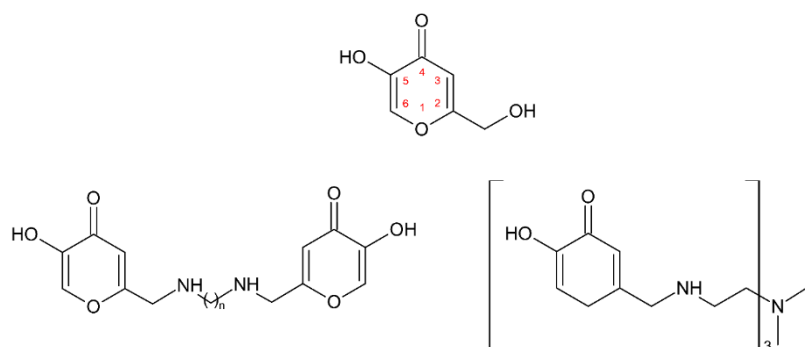
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## A FAMILY OF KOJIC ACID DERIVATIVES FOR THE REMEDIATION OF $Pb^{2+}$ AND $Cd^{2+}$ : COMPLEX FORMATION EQUILIBRIA STUDIES

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Nowdays, the urbanization and industrialization processes have increased the heavy metals exposure for humans and the accumulation into the atmosphere, waters, soils etc. Lead and cadmium are considered two of the most toxic metals with any biological function and homeostasis mechanism. However, the heavy metals toxicity depends on contaminated species, specific metal, concentration and pH. Many methods have been developed for the treatment of toxic metal ions, such as chelation therapy. In the frame of this work, the use of chelating agents would be an efficient and successful strategy for the remediation of  $Pb^{2+}$  and  $Cd^{2+}$ . We propose the use of chelating agents, S2, S3, S4 and SC (Fig. 1), based on derivatization of kojic acid (KA), by the reaction between -OH group in position 2 of KA and simple linear diamines of variable length (ethylene diamine, propane-1,3-diamine, and butane-1,4-diamine). Potentiometric and spectrophotometric titrations at 25°C and 0.1 M ionic strength allow us to calculate complex formation constants, taking into account hydrolysis process, and to evaluate the formation of binuclear complex (starting around pH 4 and pH 5 respectively for  $Pb^{2+}$  and  $Cd^{2+}$ ) where each of two  $Pb^{2+}$  or  $Cd^{2+}$  ions are coordinated by the binding oxygen atoms of KA units of two different ligand units.



**Figure 1** Ligand structures: top) kojic acid; bottom) S ligand where  $n = 2$  in S2,  $n = 3$  in S3 and  $n = 4$  in S4 on the left and SC ligand on the right.

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**PYES – AN OPEN SOURCE SOFTWARE FOR THE COMPUTATION OF IN SOLUTION AND PRECIPITATION EQUILIBRIA**

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Among all the fields where chemistry intersects with informatics, analytical chemistry is certainly of particular interest. Following a survey conducted among the participants of the Network for Equilibria and Chemical Thermodynamics Advanced Research, COST ACTION – NECTAR CA18202[1] it has been noted how scarce and inadequate the available software is. It was therefore decided to re-write ES4, a freeware computer program originally written by Prof. Silvio Sammartano from the Università degli Studi di Messina and his co-workers in the last century using the BASIC programming language [2,3], created with the aim of solving chemical equilibria in solutions. Our main goal was to produce PyES: a new, open-source, practical, modern and multi-platform Python application. Currently the software has two work modes: titration simulation and species distribution at different concentration of one of the component present (such as at different pH values). PyES is also able to manage solid species and to account for variable ionic strength of the medium, refining the formation constants by an expanded Debye-Hückel equation. The original terminal-based interface has been recreated in a more modern graphical form with graphing capabilities and the possibility of exporting the obtained results in formats that allow interoperability with other applications for further data elaboration.

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**HIGHLY SELECTIVE DETERMINATION OF METHYLMERCURY IN BIOLOGICAL SAMPLES: AN ICP-MS METHOD BASED ON THE ON-LINE BLOCKING OF INORGANIC MERCURY**

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Mercury and its species pose a major risk to all living organisms within our biosphere. Environmental behavior, bioavailability, bioaccumulation, and health effects are species-specific, with methylmercury (MeHg) showing higher toxicity than inorganic mercury (iHg). These substantial differences make the accurate and selective quantification of MeHg a priority task. In this context, we demonstrated the potentialities of a simple speciation method based on ICP-MS to determine MeHg selectively and rapidly in biological samples (fish tissue and plankton) [1]. We were able to reach this goal by pushing to the limit the frontal chromatography approach that we successfully proposed for As(III)/As(V) and Cr(VI) determination [2,3], i.e., by blocking iHg species in a short home-made column filled with a strong anion exchange resin placed in front of the ICP nebulizer. The analytical protocol involves the ultrasonic-assisted extraction of Hg species in 5 M HCl (15 min) and then, after proper dilution, the analysis of the extracting solution by ICP-MS. As an advancement, we have developed a new extraction media to avoid the dilution step: an optimized HCl-HBr-Thiourea mixture was used and directly analyzed after extraction. MeHg is determined in 100 seconds with a limit of detection of 8 ng kg<sup>-1</sup> in the solution (~ 0.4 µg kg<sup>-1</sup> in solid samples) and negligible iHg interference: iHg:MeHg ratios up to 50 (an unusual case for biological samples) are well tolerated under optimized conditions (quantification error < 15%). The overall procedure was validated by standard reference materials of human hair, phytoplankton, fish muscles and liver. Application to the marine trophic chain of Djibouti highlighted a high trophic magnification factor (TMF = 13.5) for MeHg [1] whereas the application to human hair samples showed a good correlation of MeHg concentration with the individual weekly intake of fish and seafood. These findings testify the capabilities of this method for high-throughput routine application and its low-cost and ease of implementation may contribute to the enforcement of Hg regulation.

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**THERMODYNAMIC BEHAVIOR AND SENSING PROPERTIES OF TWO CARNOSINE-BASED DERIVATIVES**

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L-carnosine (CAR), an endogenous dipeptide, plays a series of biological roles and benefits from several ergogenic and therapeutic properties. It also shows an effective chelating ability towards bivalent metal cations [1], confirmed by potentiometric studies on CAR-Cu<sup>2+</sup>, -Mn<sup>2+</sup>, -Zn<sup>2+</sup>, -Pb<sup>2+</sup>, -Hg<sup>2+</sup> systems, in NaCl aqueous solution, at different ionic strengths ( $0.1 \leq I \leq 1 \text{ mol L}^{-1}$ ) and temperatures ( $15 \leq t \leq 37 \text{ }^\circ\text{C}$ ). On the basis of the coordination capacity of carnosine, we have recently synthesized two CAR-based derivatives: (i) Ferrocenyl-carnosine (FcCAR) and (ii) Pyren-carnosine (PyCAR), in order to evaluate their possible employment as metal probes. For both systems, acid base properties and complexing abilities towards divalent metal cations were studied in NaCl solution at 25 °C and  $I=0.15 \text{ mol L}^{-1}$  by potentiometry and UV-vis spectroscopy. The sensing properties of FcCAR and PyCAR towards metal cations were investigated by voltammetry on bare and modified Glassy Carbon electrodes (GCEs), and UV-vis spectroscopy, respectively. In addition, to improve the performances of the voltammetric sensor, a homogeneous dispersion of Multi-Walled Carbon Nanotubes functionalized with Amino Cyclodextrins (MWCNT-CDs) has been immobilized, by drop-casting, on GCEs [2]. Since both systems have already highlighted promising performances, these results lay the groundwork for the design of sensors able to be exploit in the uptake of metal cations.

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## CHELATING PROPERTIES OF THE FUNGAL METABOLITE HARZIANIC ACID TOWARD TOXIC HEAVY DIPOSITIVE CATIONS ( $\text{Cd}^{2+}$ , $\text{Co}^{2+}$ , $\text{Ni}^{2+}$ , $\text{Pb}^{2+}$ )

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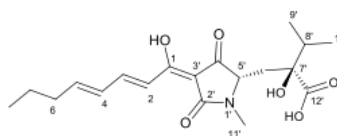
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Harzanic acid is a secondary metabolite belonging to the subgroup of dienoyl tetramic acids, essentially produced by fungi of *Trichoderma* genera. This metabolite is particular regarded for its valuable biological



Structure of harzanic acid

activities (e.g. antimicrobial, plant growth-promoting<sup>1</sup>) which are also related to its chelating properties. In this work, the complexing properties of harzanic acid toward some dipositive toxic heavy metals ( $\text{Cd}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Ni}^{2+}$  and  $\text{Pb}^{2+}$ ) have been studied because they might have a crucial role in the tolerance of plants growing in metal-contaminated soil. The measurements have been investigated at 25 °C in  $\text{CH}_3\text{OH}/0.1 \text{ M NaClO}_4$  (50/50 w/w) as ionic medium, in according to the low solubility of this metabolite in water (less than  $10^{-6} \text{ M}$ ), using techniques as UV-Vis, potentiometry, CD-UV as well as MS and NMR. In particular, the elaboration of UV-Vis data by numerical procedure<sup>2</sup> has shown the presence of neutral and negatively charged coordination complexes where metal/harzanic acid ratio is 1:1 or 1:2 confirmed also by MS and NMR measures. In addition by <sup>1</sup>H-NMR spectra analysis has been established the involvement in the coordination complexes of the C=O group of the amide and the external carbonyl groups of the ligand.

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**THE IMPACT OF METAL COORDINATION ON CALCITERMIN ANTIMICROBIAL PROPERTIES**

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Calcitermin (VAIALKAAHYHTHKE) is a 15-mer antimicrobial peptide found in human nasal fluid [1] which is of particular interest thanks to its metal chelating ability. Noteworthy, it exhibits improved antifungal (*C. albicans*) and antibacterial properties in the presence of Zn<sup>2+</sup> and Cu<sup>2+</sup> ions under acidic conditions, while the histidine-to-alanine mutation in position 9, 11 and 13 can modulate the activity against certain microorganisms [2].

In light of the above, we decided to extend the study to other calcitermin derivatives, where the amino acid sequence is modified to understand the impact of metal coordination on the antimicrobial activity. The new synthesised derivatives include the C- and/or N- terminal protected peptides, which could also decrease the susceptibility towards exopeptidases, the alanine-to-serine mutants (A7S, A8S and A7/8S), which are designed to stabilize copper complexes [3] and the truncated analogue Ac-AHYHTHKE-NH<sub>2</sub> to verify if the metal coordination site of natural calcitermin can correspond to the minimum active sequence.

This work is therefore aimed at characterizing the interaction of Zn<sup>2+</sup> and Cu<sup>2+</sup> ions with calcitermin derivatives in aqueous solutions. A deep investigation of the thermodynamic parameters of complex formation equilibria and of the coordination chemistry of the formed species has been obtained by means of several techniques, including potentiometry, high-resolution mass spectrometry, UV-Vis, circular dichroism and EPR.

Financial support of the Polish National Science Centre (UMO-2020/37/N/ST4/03165) and of the COST Action CA18202, NECTAR – Network for Equilibria and Chemical Thermodynamics Advanced Research is gratefully acknowledged.

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**METAL-COORDINATED ASSEMBLIES BASED ON QUERCETIN: SOLUTION EQUILIBRIA ARE CRUCIAL FOR DEVELOPING PH-RESPONSIVE DRUG DELIVERY SYSTEMS**

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Stimuli-responsive drug-delivery systems (DDSs) based on polymers have attracted a great deal of attention as they allow to overcome the drawbacks of some chemotherapy drugs (low solubility, poor permeability and short biological half-life) and enable their temporally and spatially controlled release [1,2].

In this work, the study of multiple equilibria in solution is proposed as the key strategy for the design and development of stimuli-responsive DDSs which take advantage of multiple non-covalent interactions involving polyacrylic acid (PAA), a biocompatible and pH-responsive polymer, metal ions of biological interest ( $\text{Cu}^{2+}$  and  $\text{Zn}^{2+}$ ) and quercetin (Que), a flavonoid with neuro/cardioprotective, anti-inflammatory and anti-cancer effects [3]. The quantitative analysis of the species, binding affinity and thermodynamic parameters for the formation equilibria involving all the DDS components has been conducted in aqueous solution at 25 °C and physiological pH by combining UV-Vis spectrophotometry and isothermal titration calorimetry (ITC) experiments. Although free Que and PAA are unable to interact, the presence of the metal ion allowed for their binding and thus its features resulted to be crucial for the formation of the final three-components assembly.

Kinetic studies provided significant insights on the stability of the metal-coordinated assemblies in aqueous solution. Finally, the release of Que from the metal-based DDSs was investigated at 37 °C and at different pH values through dialysis and UV-Vis experiments. The cumulative release % of Que allowed us to determine the delivery mechanism under various pH conditions. Overall, the results provided a detailed picture of the formation thermodynamics and mechanisms of the assemblies containing Que, which are essential for the rational design and optimal performances of these metal-based DDSs in solution.

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**IDENTIFICATION, CHARACTERIZATION AND PRESUMPTIVE TEST DEVELOPMENT OF A NEW SYNTHETIC CANNABINOID IN FORESIC CASEWORK**

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Community legislation defines NPS as a «substance in its pure state or contained in a preparation not covered by the United Nations Single Convention on Narcotic Drugs of 1961, modified by the Protocol of 1972, or by the Nations Convention on Psychotropic Substances of 1971, but which may be a risk health or social risks similar to those presented by the substances contemplated by these conventions». NPS refers to a very large group of molecules, mainly of a synthetic nature, characterized by pharmacological and toxicological properties. Analytical identification of NPS is challenging today, due to the miss of analytical standards, literature and reference methods. The structural groups known so far are synthetic cannabinoids, synthetic cathinones, opioids, tryptamines, piperazines, arylcyclohexylamines, phenethylamines but targeted molecular modifications are able to confer new structures both from a chemical-toxicological and legal point of view. Lately, the chemistry section of the Messina RIS reported the probable identification of a new molecule related to a recent seizure of plant material which took place in the Province of Messina. Routine analyses, which involve the use of GC / MS, revealed the presence of an unknown substance.

Here, we report the complete NMR characterization of a new synthetic cannabinoid, belonging to a class of compounds never identified in Europe. The structure of compound, was unequivocally assigned by integrated <sup>1</sup>H-, <sup>13</sup>C-, 2D-NMR (COSY, HMQC and HSQC). Further, matching with MS/MS fragmentation pathway analyses was carried out.

The new cannabinoid, a derivative belonging to the family [1] of the isatin analogues, was identify for structural characteristics. Moreover, a presumptive test was development in order to rapid identification *in situ*.

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**MULTICLASS ANALYSIS OF PSYCOACTIVE SUBSTANCES FROM HAIR BY PARALLEL ARTIFICIAL LIQUID MEMBRANE EXTRACTION AND UPLC-MS/MS DETECTION**

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Hair analysis is widely diffused to investigate the use of illicit drugs in forensic toxicology applications; however, extracting drugs from hair is usually a long and tedious procedure, requiring up to 18 hours and significant amounts of organic solvents [1]. Extraction is then followed by a clean-up step prior to instrumental analysis which is generally carried out by liquid chromatography coupled to mass spectrometry (LC-MS(/MS)). Clean-up typically involves classic solid and liquid phase extraction, but miniaturized techniques are gaining more interest.

Parallel artificial liquid membrane extraction (PALME) is a liquid phase microextraction involving two 96-well plates, which respectively contain the donor and the acceptor phases; between the plates a supported liquid membrane (SLM) is located. The principle of PALME is to transfer the basic drugs from the donor phase to the acceptor phase by exploiting a pH gradient. In this study we demonstrated that PALME is suitable to combine hair extraction and clean-up in a single step, reducing the consumption of chemicals (only 3µL of decanol is used in the SLM), and making this method an innovative green approach in the field of analytical chemistry [2].

Eighty basic drugs, both traditional ones and new psychoactive substances (NPS) were considered and UPLC-MS/MS was used for analysis. A Design of Experiment (DoE) was performed to find the best conditions for the donor phase, SLM, and acceptor phase. The validation of the method is still in progress.

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**DETERMINATION OF CHIRAL PESTICIDES IN HEMP SEEDS WITH SUPERCRITICAL FLUID CHROMATOGRAPHY**

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Foods can contain unintentional additives, molecules potentially toxic to human health, including pesticides. Pesticides are toxic substances that present a wide difference in both molecular structure and chemical characteristics. Many commercial pesticides (approximately 25% of the existing agrochemicals) are characterized by one or more chiral centers. It is well-known that enantiomers of a chiral molecule can possess different activities, whatever they may be. Moreover, the degradation of pesticide enantiomers in the environment can also be enantiospecific.

Hemp (*Cannabis sativa* L.), considered as novel foods, is actually used as food ingredient and can thus represent a source of pesticide.

The present research is focused on the development of an environmentally friendly, on-line analytical method - supercritical fluid extraction-supercritical fluid chromatography-triple quadrupole MS - for the extraction and enantioselective determination of chiral pesticides in hemp seeds. This is an on-line approach for the simultaneous extraction and determination of chiral pesticides. In such a respect, metalaxyl, benalaxyl and dimethenamid were searched for in nine hemp seed samples belonging to four varieties of *Cannabis sativa*. The figures-of-merit determined were linearity, precision, limits of detection and quantification.

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**DEVELOPMENT OF BIODEGRADABLE SAMPLING DEVICES FOR THE ANALYSIS OF ILLICIT DRUG IN ORAL FLUIDS.**

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Psychoactive substances consumption over the years is continually increasing; around 29% of adults in the EU are estimated to have used illicit drugs at least once in their lifetime. In order to assess the active consumption of these substances, it is essential to detect the target analyte (active principle or metabolite) in the biological matrices. Among the different matrices, oral fluid (OF) is an interesting matrix due to its simple and noninvasive sample collection and the good correlation with blood concentration, allowing the detection of recent drug intake [1]. The commercial collection devices for OF, which are typically made of plastic materials, may give incomplete desorption of lipophilic drugs or metabolites thus affecting the real concentration of the target analytes in OF. Furthermore, they are generally destroyed through open burning and incineration, resulting in the emission of dioxins, furans and particulate matter. In the last years, billions of tampons were used and measures to prevent this growing waste are desirable [2,3].

For these reasons, the goal of this work was the development of a new biodegradable sampling device. To date, new soluble and biodegradable sampling devices made with polylactic acid (PLA) and printed with 3D technology have been developed for forensic toxicology applications. PLA can be safely introduced in the mouth, as verified with IR spectrophotometry tests, and easily dissolved in acetonitrile at 50°C. The new device was tested for the simultaneous analysis of natural and synthetic illegal drugs. After the dissolution of the sampling device a clean-up is necessary, so a dispersive liquid-liquid microextraction was performed in order to clean-up and concentrate the sample before the LC-MS/MS analysis. Same procedure was applied to existing collection devices, i.e. Quantisal and Cliniswab, in order to compare the efficiency of the new developed device. Preliminary results are promising, so once optimized the protocol will be validated according to national and international guidelines.

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**GC-FTIR AFFORDS RELIABLE FORENSIC EVIDENCE FOR DRUGS OF ABUSE**

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Drug exhibits have become more challenging over the years due to an increase in complex synthetic substances with many positional isomer and diastereomer possibilities.

Infrared spectroscopy is able to readily provide detailed chemical information for differential analysis at a higher level of specificity and discrimination that is often unattainable through the use of tandem or high-resolution MS techniques.

Coupling the separation power of gas chromatography with the identification ability of infrared spectroscopy affords complementary information based on the retention behavior of the analytes and their spectral fingerprints.

This presentation will introduce solid phase deposition GC-FTIR as a technique which allows for improved specificity and reduced uncertainty in identification of regioisomers, diastereomers, analogs, and other closely related compounds such as fentanyl analogs, cathinones, phencyclidines, and synthetic cannabinoids.

**EXPOSURE ASSESSMENT OF NITRITE AND NITRATE INTAKE FROM LEAFY VEGETABLES CONSUMPTION THROUGH MARGIN OF SAFETY (MoS) EVALUATION**

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Nitrite and nitrate levels in leafy vegetables are a matter of concern since nitrate accumulates so much in these foods and some authors also reported high nitrite levels. In this study, 274 samples of leafy vegetables samples (spinach, lettuce, chard and wild rocket) were analysed using ion chromatography for determining nitrite and nitrate. Low and high exposure scenario were considered for each product type using the reference data available from the INRAN-SCAI 2005-06 Italian National surveys on food consumption. Nitrite and nitrate are not genotoxic/carcinogenic, so, the Margin of Safety (MoS) was evaluated, using 1 as the minimum requirement and 100 as a protective threshold. The MoS was calculated as the Admissible Daily Intake (ADI)/Estimated Exposure Dose ratio, for toddlers and adults, considering 12 and 70 kg as reference b.w., respectively, as established by EFSA. Regarding nitrite, 4 MoS values were lower than 1 under high exposure scenario. All these values were related to toddlers population and were obtained for fresh-cut spinach and chard (0.8 and 0.9, respectively), frozen spinach (0.4) and lettuce (0.7). Regarding the protective MoS value of 100, only 8 values out of 38 (21%) resulted higher. Obviously, the general framework is more reassuring for adults unless a very low value, worthy of attention, was obtained for frozen spinach consumption under high exposure scenario (2.6). Regarding nitrate, only 1 MoS value was lower than 1 (fresh lettuce, toddlers, high exposure: 0.5). Moreover, it is worth mentioning that 2 MoS values calculated for toddlers under high exposure scenario were close to 1: fresh-cut spinach and chard (1.6 and 1.1, respectively). Regarding the protective MoS value of 100, 9 values out of 40 (22.5%) resulted higher. The results suggest both the introduction of legal limits for nitrate in Swiss chard and nitrite in leafy vegetables, and the intensification of official controls, especially regarding the levels of nitrate in wild rocket (11 samples out of 44 analysed with nitrate concentration higher than the EU limit). Another significant remark is the greater mean concentration of both nitrite and nitrate in fresh-cut samples. This finding suggests the development of further research aimed at identifying and evaluating the causes of such increases in these products.

This work was supported by the Italian Ministry of Health (Project code IZSPB 07/20 RC).

**UNVEILING THE OXYGEN HETEROCLYCLIC FRACTION OF CITRUS SCENTED HAND GEL SANITIZERS BY MEANS OF HPLC-MS/MS: FOCUS ON COUMARINS AND FUROCOUMARINS CONTENT AND SKIN RISK TO EXPOSURE.**

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During the last two years, the pandemic has posed serious challenge in develop effective method to reduce Covid-19 spread. World Health Organisation (WHO) define Alcohol-Based Hand Rub (ABHRs) as “*an alcohol-containing preparation (liquid, gel or foam) designed for application to the hands to inactivate microorganisms and/or temporarily suppress their growth*” and suggest their use as effective alternative to handwashing. EU legislation define hand gels as cosmetic or biocide. They can be named as “hand sanitizer” when ethanol content and biocidal claim are reported in the label. Conversely, cosmetic gel do not fit for use as disinfectants and a more appropriate denomination would be “hand cleanser” or “hand gel”. Today, hundreds of ABHRs are available on the market with various combinations of ingredients. A frequent modification to the formulation is the use of fragrances to cover for alcohol strong odour. However, fragrances can be irritating to those with sensitive skin or can cause allergic reactions. To this regard, when citrus essential oils are added to cosmetics, coumarins and psoralens content should be disclosed. Indeed, the European Regulation *EC N°1223/2009*, establish the maximum amount of psoralens allowed in cosmetics, and *International Fragrance Association - IFRA*, have issued Opinions based on studies related to psoralen induced phototoxicity and skin damage.

In this research, twelve ABHRs scented with lemon, orange, bergamot, and grapefruit and a gel that does not contain fragrances, were tested. The OHCs fraction was extracted in Ethyl Acetate and analysed using a HPLC-MS/MS instrument operating in Multiple Reaction Monitoring (MRM). The method allows the simultaneous quantification of 37 compounds enabling also the rapid identification of those products in which the OHCs profile did not match the one corresponding to the citrus oil reported in the label.

**A MICROFLUIDIC PAPER-BASED DEVICE FOR ESTIMATING THE POST-MORTEM INTERVAL (PMI): AMMONIUM ANALYSIS IN VITREOUS HUMOR**

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The estimation of the post-mortem interval (PMI) is a crucial question that forensic experts need to answer directly at the crime scene to carry out their investigations effectively. There are various on-site approaches to estimating the PMI, but they rely on data collected from the body inspection. In order to obtain more accurate and objective data, the analysis of vitreous humor (VH) for estimating the potassium concentration must be carried out. A recent study revealed that the concentration of ammonium in the VH could also be correlated to the PMI [1]. However, these approaches require expensive laboratory equipment.

Since the first report about the use of paper-based devices ( $\mu$ PADs) [2], several devices for forensic chemistry aims have been proposed [3]. The devices are obtained by patterning chromatography paper into hydrophilic channels by fabricating hydrophobic barriers and adding a specific reagent to a hydrophilic portion of the device. The capillarity action of the paper allows the sample to reach the reagent zone without external assistance. The easiest approach allows detecting the analyte as a result of a colorimetric reaction.

This presentation aims to show the use of a  $\mu$ PAD for detecting ammonium in VH. The device was designed to generate a first-line response for inferring the PMI on-site.

The proposed device was based on a selective colorimetric reaction between ammonium and the Nessler's reagent. The device was able to detect ammonium in VH down to 0.4 mmol/L, showing trueness within 94.5-104.5% and a precision lower than 9.3%. The  $\mu$ PAD was successfully used to determine the ammonium in VH samples (n=25), showing a close correlation with the results obtained with capillary electrophoresis ( $R > 0.88$ ). The results indicated that the developed  $\mu$ PAD could be used as an additional tool to estimate the time of death at the crime scene [4]. The proposed device has the potential to offer a real breakthrough thanks to the capability to obtain an on-site rapid and objective estimation of the post-mortem interval.

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**ANALYTICAL PYROLYSIS-GAS CHROMATOGRAPHY  
COUPLED WITH MASS SPECTROMETRY (Py-GC/MS) TO  
CHARACTERISE LIGNIN EXTRACTED WITH DEEP EUTECTIC  
SOLVENTS**

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Deep eutectic solvents (DESs) are a novel class of solvents with interesting potential to extract lignin from lignocellulose [1]. The extraction efficiency of DESs is usually evaluated with gravimetric yield, and the extracted lignin is typically characterised using spectroscopic methods. On the contrary, mass spectrometry-based techniques are far less used, despite their potential to provide information on the features of DES-lignin at a molecular level.

Here, we present a method to extract lignin from fir wood using choline chloride/formic acid DES. Microwave heating was also used to enhance the efficiency of the extraction process and to reduce the extraction time. The method was optimised with a Box-Behnken experimental design.

Analytical pyrolysis-gas chromatography coupled to mass spectrometry (Py-GC/MS), a well-known and powerful technique to characterise lignocellulose, was used to evaluate the extraction efficiency and the structural features of extracted lignin. In addition to the gravimetric yield, two new parameters were calculated by performing semi-quantitative analyses on the Py-GC/MS profiles of both the extracted lignin and the cellulosic residue. The peak areas ratio of lignin-specific to holocellulose-specific pyrolysis products in the cellulosic residue (L/H ratio) was used to estimate the amount of residual lignin on the substrate after the extraction, while the percentage peak area of guaiacyl lignin monomers in the extracted lignin (%G) was used as indicator of the structural integrity.

Mild extraction conditions (100 °C, 15 min) provided high L/H of the residue, indicating poor extraction efficiency, but the extracted lignin showed high %G, suggesting that the original structural integrity was preserved. On the other hand, harsh extraction conditions (150 °C, 45 min) provided opposite results, favouring a high extraction yield at the expense of a significantly reduced structural integrity.

The response models based on Py-GC/MS data provided better fittings than the one based on gravimetric yield, as they were less affected by the presence of impurities and sub-products of the extraction process. These results highlight the potential of Py-GC/MS in evaluating the extraction efficiency and the structural features of DES-lignin.

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**DETERMINATION OF PESTICIDES IN FOOD SAMPLES THROUGH REDUCED SAMPLE PREPARATION COUPLED TO FLOW- AND CRYOGENIC-MODULATION GC×GC COMBINED WITH TRIPLE-QUADRUPOLE MASS SPECTROMETRY**

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The present research is focused on the determination of pesticides in food samples by using reduced sample preparation coupled to flow and cryogenic-modulation comprehensive 2D GC combined with triple-quadrupole mass spectrometry (GC×GC-QqQMS). Specifically, 88 pesticide residues in four vegetable products and 70 pesticide residues in oil samples were determined. Vegetable samples were analyzed using a “reduced-scale” QuEChERS extraction method and flow-modulated GC×GC-QqQMS. While the oil samples were only treated with limited volumes of acetonitrile (500 µL), analyte introduction onto the first-dimension column was performed through a programmed-temperature vaporizer (PTV). The exploitation of cryogenic modulation and QqQMS (in the multiple-reaction-monitoring mode) eliminated the need of a target-analyte concentration step. Furthermore, the use of two analytical columns with a high thermal stability and with a thin film thickness, enabled the elution of high boiling-point matrix interferences. The GC×GC-QqQMS methods developed were demonstrated to be suitable for the trace level determination of food samples contaminants, in relation to maximum residue limits set by the European Union.

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**DEVELOPMENT OF AN ADVANCED EXTRUSION PROCESS FOR THE REDUCTION OF VOLATILE AND SEMI-VOLATILE ORGANIC COMPOUNDS OF RECYCLED HDPE FROM FUEL TANKS**

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Plastic fuel tanks of vehicles are typically made of high-density polyethylene (HDPE). The easiness of the dismantling procedure from end-of-life vehicles and the intrinsic recyclability of HDPE allow them to be accounted in principle in the “white list” of recyclable components. However, the strong contamination and the odor produced by the volatile (VOCs) and semi-volatile (SVOCs) organic compounds, which have been absorbed during the service life, drastically hinder the use of this end-of-life material. This aspect reduces its actual recyclability, especially in higher-value applications. The aim of this scientific work is the achievement of an innovative extrusion process, specifically designed for the stripping out of these organic contaminants. An in-depth analytic approach is also reported to identify their nature and fraction, as a way for ranking the effect of the different processing conditions which have been tested. The developed extrusion process uses a co-rotating twin-screw extruder with degassing points and the injection of water as medium for desorbing the organic contaminants. The analytic approach is based on headspace (HS) sampling associated with gas chromatography coupled to a mass spectrometric detection (GC-MS) [1]. The multivariate approach of the Principal Components Analysis (PCA) is applied on the entire dataset collected in the experiments, including the HS-GC-MS data, the different process parameters and the mechanical and thermal data. As a result, the effect of the process conditions and all the organic contaminants present in the material are identified. The developed extrusion process allows to obtain a material with higher opportunity to be used in applications which require enhanced performance and, therefore, to be considered effectively recyclable, further reducing the environmental impact of the end-of-life vehicles.

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**DEVELOPMENT OF A PROTOTYPE SYSTEM BASED ON HIGH TEMPERATURE LIQUID CHROMATOGRAPHY FOR THE DETERMINATION OF PARABENS IN COSMETIC AND FOOD SAMPLES**

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Parabens are esters of p-hydroxybenzoic acid; these compounds are chemicals commonly used as artificial preservatives in various products, like foods, personal care products, pharmaceutical drugs, and beverages. [1]

The most commonly used parabens are methyl- (MeP), ethyl- (EtP), propyl- (PrP), and butyl-parabens (BuP).

The high antibacterial activity in a wide pH range, the low cost, the thermostability, the odorless, the tasteless, have attracted the interest of researchers for these compounds. In this work, we developed and validated a green workflow for the extraction, separation and quantitation of target analytes, using only water and ethanol as solvents. To this regard, separation techniques, including mainly Liquid Chromatography (LC), involves the consumption of large amounts of organic solvents. In LC, an ideal mobile phase should possess many requirements, such as: the ability to dissolve ionized, polar and nonpolar analytes; no flammability; nontoxicity; high grade of purity; low viscosity to generate low backpressure; high diffusion rate; and low cost. Most organic solvents fail on a number of these criteria, especially low cost, toxicity and flammability.

This issue can be overcome by replacing the organic solvent with pure water at high temperature, making more eco-sustainable the developed analytical method. In order to realize this kind of application, the availability of LC column resistant at high temperature is mandatory. Recently, porous graphitic carbon was proposed as an alternative to C18 phase, since it provides significant retention for both non-polar and polar analytes [2]. In this work, the performances of two carbon columns were evaluated, in terms of robustness, efficiency and resolution. Finally, the method was validated according to Eurachem guidelines.

## GREEN-OR04

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## A SUSTAINABLE STRATEGY FOR THE PRODUCTION OF BIOETHANOL FROM CITRUS WASTE

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A sustainable strategy for the exploitation of citrus fruits processing waste (CW) aiming at the obtainment of bioethanol (BioEt) by fermentation, has been designed and implemented. Process innovation mainly consisted of maximizing both eco-efficiency and yield. The experimental plan has been applied to samples of orange and mandarin waste, preliminarily deterpenated (i.e. limonene-free), and coming from Sicilian citrus processing plants. The conventional process applied to the production of BioEt has been redesigned with a “green vision”, thus overcoming its weakest points such as low yield and high environmental impact<sup>1</sup>. In particular, key steps of our approach have been focused on: i) selection of optimal bacterial strains; ii) substitution of acidic hydrolysis treatment with physical and mechanical methods; iii) increase of final bioethanol yield through circular recovery of residual dry matter. The green protocol applied to sample pretreatment, based on autoclaving and sonication followed by enzymatic hydrolysis using  $\beta$ -glucosidase, pectinase and cellulase, has allowed the achievement of an excellent yield in reducing sugars (79.5% for orange mash; 64.1% for mandarin mash). The alcoholic fermentation of the pre-treated samples was conducted in bioreactor, by inoculating the selected bacterial strains *S. cerevisiae* ATCC 9763 and *S. bayanus* BCS103, under controlled pH, temperature, time, and pressure conditions. From the fermentation broth, the liquid residue (supernatant) and the dry residue (pellet) were separated. The first has been centrifuged and steam distilled for the extraction of BioEt; the second has been replenished circularly in the process for further extraction of BioEt. This circular reclaim has positively impacted on the final product yield, exceeding 15 g/kg of biomass for both types of waste treated.

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**COMPARISON OF RETENTION BEHAVIOR OF NATURAL CANNABINOIDS IN SUPERCRITICAL FLUID CHROMATOGRAPHY AND NORMAL PHASE LIQUID CHROMATOGRAPHY**

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One of the chromatographic techniques that can be used for the resolution of mixtures of enantiomers is high performance liquid chromatography under normal phase conditions (NP-HPLC). This technique is very useful when the mixtures to be separated are not soluble or have small solubility in water. In NP-HPLC the mobile phase consists of a mixture of a non-polar solvent (e.g., Hexane or Heptane) and an alcohol. However, hexane is a highly volatile and dangerous solvent as it is metabolized in humans, causing damage to neuro-cerebral system; for this reason, there has been a growing concern related to the use of this solvent [1]. Continuous exposure to hexane has been shown to cause both loss of sensory and motor functions. Supercritical Fluid Chromatography (SFC) could be the alternative candidate technique to the use of NP-HPLC. SFC has only been widely used in some specific fields, but the use of this technique has grown in recent years both for the instrumental advances but also for its green characteristics. SFC is considered environmentally friendly as it minimizes the use of toxic and hazardous solvents [2]. The most used mobile phase is supercritical CO<sub>2</sub>, which in some processes can be recycled in the chromatographic system. The system works with the same mechanism of normal phase chromatography. In SFC, CO<sub>2</sub> is the main component of the mobile phase and organic modifiers are added to the mobile phase to increase the solubility of the compounds but also to induce selectivity variations due to the formation of weak interactions such as hydrogen or dipole-dipole bonds [3]. One of the applications of SFC is the separation and isolation of complex natural mixtures such as the phyto-cannabinoid class. The determination of the composition and therapeutic properties of the *Cannabis sativa* L. plant have become a critical point of study due to the increasing use of this class of compounds in the medical field. The number of natural cannabinoids identified is over 100, most of which are chiral, but in most cases the minor cannabinoid enantiomers are not available as commercial standards [4]. There are no comparative studies in the literature on the fundamentals of cannabinoid retention under SFC and NP conditions. These studies are fundamental to show that it would be possible to replace NP-HPLC with SFC, so as not to use toxic solvents, performing faster runs, etc. In this work, the retention behaviour of different cannabinoids was evaluated under both SFC and NPLC condition, using Whelk-O1 columns packed with sub-2 µm fully porous particles. The study also evaluated the possibility of using an innovative approach called Inverted Chirality Column Approach (ICCA) which

makes use of two chiral stationary phases (CSPs) having the same selector but with an opposite configuration i. Using SFC promising results have been obtained, the separation of the main cannabinoids using CO<sub>2</sub> as mobile phase is comparable to that obtained with normal phase liquid chromatography and, in some cases, variations in selectivity have been observed.

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**GREEN EXTRACTIONS OF AROMATIC HERBS AND CITRUS WASTE: NOVEL CONFIGURATIONS FROM LAB TO PILOT PLANT SCALE**

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Green extractions aim at the full valorization of aromatic herbs along with the minimization of energy consumption, extraction time and cost of the processes. Ecofriendly approaches like microwave assisted extraction (MAE), ultrasound assisted extraction (UAE) and solventless MW-assisted extraction aim to exploit waste materials recovering essential oils (EOs), hydrosols and solid residues as valuable products. Here, we present the results on the full valorization of aromatic herbs (i.e. lavender and rosemary) and citrus waste (orange peels) by exploiting the different fractions (EOs, hydrosols and solid residues) obtained by MAE, UAE and solventless MW-assisted extraction. All the extraction configurations are based on the uses of a coaxial MW antenna, a versatile technology that benefits of having extractor vessels assembled with any material and geometry, which can be safely installed in every kind of research laboratory or industrial equipment.

The proposed green extractions are compared with the classical hydrodistillation in terms of EO yield wt%, polyphenols concentration, and thermochemical characteristics of the solid residues. All the products were characterized using different analytical techniques (head space gas chromatography mass spectrometry, HS-GC–MS, liquid chromatography with UV/visible diode array/fluorescence detector, HPLC-DAD-FD, FTIR spectroscopy in tandem with chemometrics, thermogravimetric analysis, TGA, and combustion calorimetry). The advantages of using green extractions with high yields of EOs, valuable hydrosol properties and performing energetic features of solid residues, highlight their great potential. Moreover, the pilot plant resulting from the proposed methodologies developed during the research project InSole (Agronomic and technological innovations for sustainable herbs cultivation and high quality essential oils extraction, FEASR-PSR SICILIA 2014-2020) are presented.

**THE EUTECTIC MIXTURE FORMED BY L-MENTHOL AND BUTYLATED HYDROXYTOLUENE: A GREEN ANTIOXIDANT SOLVENT FOR THE SUSTAINABLE EXTRACTION OF FAT-SOLUBLE MICRONUTRIENTS FROM RAW AND COOKED FOODSTUFF.**A. Gentili<sup>1</sup>, C. Dal Bosco<sup>1</sup><sup>1</sup>Dipartimento di Chimica, Università degli Studi di Roma “La Sapienza”, Piazzale Aldo Moro, 5 – 00185 Roma

The search for new solvents derived from natural and renewable sources is among the main purposes of Green Analytical Chemistry. In this regard, type V deep eutectic solvents (DES) are the latest trend<sup>1</sup>. In particular, depending on the selected starting materials, one can obtain both eutectic liquids showing significant negative deviation from their ideal solid-liquid equilibrium phase diagrams (DES) or ideal systems (IES). In spite of their higher eutectic temperature, the last ones can exhibit unique features, which can be very useful for some analytical applications. This is the case of the eutectic mixture here presented, composed of L-menthol and butylated hydroxytoluene in a molar ratio of 3:1 (MEN:BHT (3:1)), whose distinguishing feature is the antioxidant activity. This mixture has been successfully applied to the extraction and preservation of fat-soluble vitamins and carotenoids, most of which are photosensitive and easily oxidizable, from food samples<sup>2</sup>. In the case of fruit juices, a dispersive liquid-liquid microextraction procedure (DLLME) using MEN:BHT (3:1) as extracting solvent and ethanol as dispersing solvent has been applied, allowing a fast and even greener sample preparation. Extracts were analysed by high-performance liquid chromatography–tandem mass spectrometry (HPLC-MS). The developed method was then validated on a commercial fruit juice containing declared values of  $\beta$ -carotene and  $\alpha$ -tocopherol acetate, providing precise (4–8%) and accurate (4–6%) results. Recoveries were  $\geq 70\%$ , while the detection limits were  $0.05 \mu\text{g L}^{-1}$  for  $\beta$ -carotene and  $0.28 \mu\text{g L}^{-1}$  for  $\alpha$ -tocopherol acetate. The MEN:BHT (3:1) has been also used in the solid-liquid extraction of red pepper samples, both raw and cooked in a traditional or solar oven, with the aim of evaluating the effect of the cooking method on the nutritional value of foodstuff and developing new sustainable solar-powered ovens.

The eutectic solvent MEN:BHT (3:1) is safe and sustainable, since it is composed of cheap, low toxicity and naturally derived starting materials; moreover, the solvent can be cleaned-up after use by treating it with a low-cost carbonaceous material derived from coconut shells<sup>2</sup>.

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## GREEN-OR08

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**QUANTIFICATION OF VOLATILE MARKER COMPOUNDS IN FLAVORED CITRUS PRODUCTS BY USING SPME-GC/MS**

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In the last few years, flavored beer market showed a growing interest and the assumption of citrus flavored beers has increased significantly.

Beer production is not subject to a specific legislation as concern flavoring process. Producers are not obliged to specify which product they use as natural ingredient for the flavoring addition process (peel, juice, pulp, essential oils), and the lack of this detailed information on the labels makes these beers subject to possible adulterations [1].

Terpenes compounds and their oxygenated derivatives are the main constituents of the volatile profile of flavored citrus products; among them, some compounds have sensitizing effects and could cause possible dangerous effects for consumers' health. In this contest, the detection and quantification of volatile marker compounds in citrus flavored beer enable to identify authentic citrus flavor and discover possible frauds and avoid possible intolerance. For this purpose, several commercial and crafted citrus flavored beer were analyzed as is, and spiked with different aliquots of a mixture of the different typical citrus marker compounds to detect their content in each beer. The linear retention indices were applied as additional parameters to mass spectra. The analysis were carried out acquiring the spectra both in Full Scan mode and in Selected Ion Monitoring mode to increase sensitivity of the method, acquiring the two most abundant fragment for each terpene in a selected window range. Limit of quantification and limit of detection have been also calculated.

Despite the variables involved in the final beer composition are various and depending by different production steps, this study underline how the developed method by using solid phase microextracion and GC characterization supported by the LRI system and libraries, can be applied for food quality control and fraud prevention.

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**BIO-SMART LABELS AND CHEMOMETRICS: MAKING THE DIFFERENCE TOWARDS SUSTAINABLE FRESHNESS MONITORING**

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In the sensing field, the development of differential sensors arrays represents one of the most promising trends, thanks to the possibility of jointly exploiting digital images colourimetric analysis and multivariate data elaboration. [1] Among the various applications, food freshness monitoring poses an interesting challenge due to the spoilage processes complexity, the key requirements for this kind of devices and the environmental and social impact. The consumers increasing interest for fresh, high-quality, minimally processed, foods has encouraged many research groups around the world to explore various strategies for the development of freshness monitoring devices, usually called intelligent packaging or smart labels. [2]

In the last years, we identified a winning approach for the development of naked-eye smart labels, focusing on solid protein foods, like fish or meats. The receptors used were pH indicators that turn their colour in a wide pH range, able to detect volatile spoilage by-products with different acid-base behaviour, while several solid supports and linkage mechanisms have been tested, ranging from ion exchanger to covalently modified polymers. [3-7]

To further increase the biocompatibility and sustainability of these devices, we developed a new drop-deposited dual sensors array, made of functionalised carboxymethylcellulose (CMC) and pure cellulose as support. The entire preparation procedure is optimised by Design of Experiments; the sensors array is fully characterised both by analytical and physico-chemical measurements. Subsequently, the developed smart label is successfully employed to assess the freshness of various poultry meat samples; the naked-eye readout is verified and various chemometric tools are applied on sensors RGB triplets to rationalise the spoilage process. Finally, the smart labels attributions are validated by independent instrumental or microbiological analysis.

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**IMPACT OF THE ANTIGEN SATURATION HOOK EFFECT ON THE DEVELOPMENT OF FIVE LATERAL FLOW IMMUNOASSAYS DETECTING INFECTIOUS DISEASES**

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The worldwide popularity of the lateral flow immunoassay (LFIA) technique has increased in recent years. LFIA, as the quintessence of the point of care (POC) testing, played a crucial role in the last pandemic [1]. The typical LFIA strip is composed by a nitrocellulose (NC) membrane where the bioreagents are dry stored in a glass fibre reservoir, and in confined areas, called test (T) and control (C) lines. The sample is added to the sample well of the device, resuspends the labelled reagents from the glass fibre reservoir and flows through the NC binding (or not) the T and C lines, giving the result (generally visual) in 10-15'. As any other immunoanalytical method, it can involve different formats, including the most important and widely used for the diagnosis of animal or human infectious diseases: the antigen test [2]. The antigen LFIA involves the so-called sandwich type immunoassay, using a capture antibody into the test line and a detection antibody able to bind the analyte (the antigen), in two different sites at the same time. Antigens are bio-macromolecules, such as viral, bacterial, or fungal proteins. Developing antigen LFIAs can be challenging, and some aspects must be considered, especially when low quantity of bioreagents are available. Generally, in this format, the most used approach is to increase the sensitivity by using large excess of bioreagent [3]. Nevertheless, exploring the potential of the involved bioreagents by means of experimental design, different behaviours between antigen LFIAs if the antibody used is the same for the capture and for the detection (single epitope, SE, sandwich), or if the two are different (double epitope, DE, sandwich) are revealed. In fact, if the most common approach could be successful when two different antibodies are available, and the DE can be used, when the SE is the sole choice, saturation phenomena and competition between the capture and the detection antibodies takes place, resulting in an overall "less is more" response of the test. We called this antigen saturation hook effect (asHE), and we proposed a model for its explanation and interpretation. We observed and studied this phenomenon

during the development of five detecting devices in which the SE was involved: Eurasian, SAT1 and SAT2 serotypes of bovine Foot-and-mouth disease [4], Lumpy Skin Disease, and African Swine Fever. In all the studied devices, the signal reporters were gold nanoparticles (AuNP) absorbed with antibodies. The signal reporters have been individuated as the most influencing actor in the asHE. The amount of antibody onto the AuNP surface, optical density of the gold conjugate, size of the AuNPs, distance between the test line and the start of the run were explored focussing on the effect on the antigen saturation.

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**SELF-POWERED ENZYMATIC AND GATE MACHINERY FOR ULTRASENSITIVE DETECTION OF HEPATITIS B VIRUS (HBV)**

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In this study, we design a new strategy for the selective detection of hepatitis B virus DNA (HBV-DNA). This approach is based on enzymatic machinery logically operated able to detect the variation of physiologically relevant biomarkers for liver dysfunctions, Alanine aminotransferase (ALT) and lactate dehydrogenase (LDH) [1]. ALT and LDH represent respectively INPUT A and INPUT B of an AND gate and produce lactate as the final output. The anodic electrode presents lactate oxidase connected in mediated electron transfer through ferrocene moieties and is able to oxidize lactate to pyruvate and to create an amplifying recycling mechanism. Instead, the cathodic electrode contains a *Myrothecium verrucaria* bilirubin oxidase (MvBOx) and modified with SiO<sub>2</sub> nanoparticles (SiO<sub>2</sub>NPs) functionalized with phenyl boronic acid and trigonelline [2]. The local pH change at the electrode surface promotes the negatively recharging of modified SiO<sub>2</sub>NPs triggering the release of two detecting DNA sequences, one labeled with fluorescein (FAM) and the other one with a dark quencher (BHQ-2), both complementary to HBV-DNA. The proposed DNA machinery represents a selective method to detect HBV-DNA (Figure 1) with an ON/OFF threshold at 0.05 fM in model solutions and could be of particular interest for future biomedical applications (e.g., early detection of liver cancer disease etc.) [3].

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**ACTIVE PRINCIPLES MONITORING IN PARKINSON'S DRUGS VIA EASY, LOW-COST AND FAST-RESPONSE COLORIMETRIC DETECTION STRATEGIES**

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The current therapeutic drug monitoring strategies do not ensure the continuous drug monitoring in patients. The development of pocket, cost-effectiveness, easy-to-use and fast-response diagnostic device could lead numerous advantages in the improvement of individualised therapy. At this purpose, we focused our work in the development of alternative detection strategies for the drug monitoring in Parkinson's patients.

Parkinson's disease is neurodegenerative disorder caused by the degeneration of dopaminergic neurons upon pH-dependent oxidative conversion of neurotransmitters like dopamine into cytotoxic molecules. Due to its polarity, dopamine cannot directly administrated since is unable to cross the blood-brain barrier. For this reason, the pharmacological therapy to treat Parkinson's patients foresees the administration of levodopa and carbidopa-based drugs. Levodopa, the main active principle of Parkinson drugs, is converted in dopamine by decarboxylase when reach the brain. Here, levodopa was firstly selectively quantified in standard solutions and in two commercial drugs formulation with very good reproducibility ( $CV_{av}\%$  3%) and very good sensitivity, with limit of quantification about  $0.6 \text{ mg L}^{-1}$ . Then, levodopa was detected also in a complex matrix as artificial urine with a good limit of quantification  $27.6 \text{ mg L}^{-1}$ . Levodopa was quantified by exploiting a colorimetric reaction that lead to the purple melanochrome formation which was here isolated and characterized through mass spectrometry techniques for the first time [1].

Carbidopa is in co-presence with levodopa. The role of carbidopa consisting into inhibit decarboxylase enzyme which would metabolize levodopa prior to reach the brain. We report also about the colorimetric determination of carbidopa by exploiting the yellow and fluorescent dimetliylamitiobenzalazine formation ( $LOD 0.41 \text{ mg L}^{-1}$ ,  $CV_{av}\%$  4) [2].

The colorimetric methods, here developed, could revolutionize the monitoring of Parkinson's drugs in patients. In addition, the proposed tests are very simple and effective appearing as a rapid and low-cost alternative to other methodologies, that usually involves large and expensive instrumentations, for drug estimation and quality control of pharmaceutical formulations.

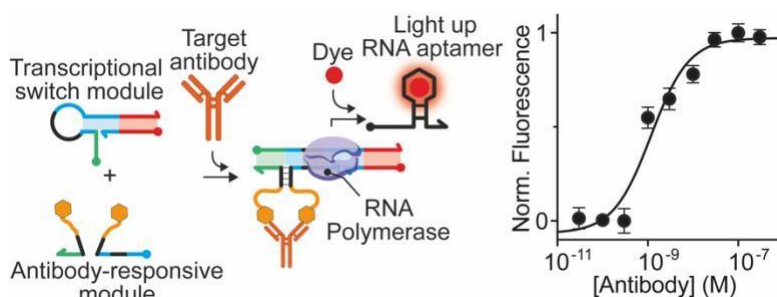
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## PROGRAMMABLE CELL-FREE TRANSCRIPTIONAL SWITCHES FOR ANTIBODIES DETECTION

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Antibody detection is important in several clinical settings because it informs on current and past infection and can provide information about clinical outcomes. Synthetic biology devices would bring new capabilities to diagnostics methods by creating sensors with new functions, expanding the range of targets, and improving sensitivity and specificity. In recent years, cell-free biosensors for the detection of nucleic acid, small molecules, and proteins<sup>1-2</sup> have been developed with excellent sensitivities and specificities. Despite the above advances, the examples reported so far of cell-free biosensors have been developed for a limited number of targets. We demonstrate here a cell-free diagnostic platform for the detection of specific antibodies directly in blood serum based.<sup>3</sup> The approach is based on the use of programmable antigen-conjugated DNA-based conformational switches that, upon binding to a target antibody, can trigger the cell-free transcription of a light-up fluorescence-activating RNA aptamer (Fig.1). The system we proposed couples the advantageous features of responsive DNA-based conformational switching probes (i.e., programmability and specificity) with those of cell-free diagnostic methods (i.e., sensitivity).



**Figure 1.** Programmable antibody-responsive transcriptional switches.

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**LASER PATTERNING FOR CONTROLLED METAL NANOSTRUCTURES FORMATION FOR SENSORS DEVELOPMENT**

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The decoration of inert and conductive substrates with nanomaterials (NMs) in the analytical chemistry field is still a hot topic. Particularly, the controlled metal nanostructures (M-NSt) decoration can allow the implementation of new sensing strategies and devices and the boosting of the existing ones. Nowadays, the challenge is the controlled M-NSt decoration onto alternative and flexible substrates (i.e., cellulosic, flexible plastics, textiles, etc.) for which often the conventional strategies result not effective or not usable.

In this presentation, different technologies to fabricate affordable and flexible optical and electrochemical nanostructured devices will be presented. In particular, will be reported the rational use of the CO<sub>2</sub> laser to form M-NSt onto cellulosic, plastic, and conductive substrates. In brief, the CO<sub>2</sub> laser by using carefully optimized conditions allows the instantaneous formation of M-NSt on different substrates in an ordered and controlled way, allowing tailored decoration geometries with high resolution.

(i) A paper-based optical device based on M-NSt of gold (Au), silver, platinum, copper, and nickel will be presented; the colorimetric device has been challenged in different applications (direct sensing, enzymatic biosensing, catalytic sensing) exploiting the different M-NSt properties, conferred by the nano structuration and metal chemistry.

(ii) Lab made electrodes were also produced based on laser decored nanostructures; in this case, the strategy relies on the instantaneous laser-induced co-reduction of graphene oxide (GO) and noble metal source (Au, Ag, and Pt) to form highly nanostructured reduced graphene oxide (rGO) conductive films integrating naked M-NSt. The hybrid films were integrated onto lab-made flexible electrodes and applied for the analysis of different analytes (phenols, nitrite, H<sub>2</sub>O<sub>2</sub>) in model solution and real samples.

(iii) Finally the preliminary results of a sintering/M-NSt decoration strategy for conductive ink will be also presented.

All the devices presented were fully Lab-made employing a CO<sub>2</sub> plotter, a craft-cutter plotter, and a thermal laminator, using office substrates. This presentation aims to demonstrate how the marriage between nanomaterials laser patterning, flexible affordable substrates, and within everyone's reach fabrication strategies represent an appealing opportunity for the development of a new generation of analytical devices.



## A 3K-TOOLBOX TO TRIGGER EPITOPE IMPRINTING INTO BIOPOLYMERS: THE CASE STUDY OF PD-L1 SENSING DETECTION

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Polycatecholamines (pCAs)-based imprinted biopolymers (IBPs) represent the new performing generation of biocompatible receptor mimetics. pCAs are intended to pave the way for “breaking old barriers”, i.e., bioreagents and classical non-sustainable polymers. Indeed, these have shown a noteworthy impact on bioanalysis in terms of reagents sustainability and cost, stability, versatility, and handling ease [1-3]. In this context, dealing with IBPs synthesis for bio-macromolecules detection/extraction, one of the critical steps in ensuring effective molecular binding affinity is the selection of suitable epitopes, short synthetic peptides, to be imprinted. To address this challenge, we designed a modular imprinting strategy to be applied when epitopes have no imprinting capability or affinity with pCAs, here focusing on polynorepinephrine (PNE). In detail, we investigated the ability of lysine (K) residues to trigger the epitope imprinting process into a PNE matrix. To this aim, we first designed a set of model epitopes composed of three K and six alanine (A) residues, permutating the K relative positions and distance along the peptide, to investigate the influence of each ‘KA’ combination on the imprinting process and the resulting binding performance *via* Surface Plasmon Resonance (SPR). Interestingly, only the case of three flanking K residues located in N-terminus arose as an excellent trigger for epitope imprinting. The efficacy of the 3K-tag-based strategy was then evaluated by exploiting epitopes belonging to soluble programmed cell death protein 1 ligand (PD-L1), a glycoprotein currently of great interest as potential cancer biomarker in liquid biopsies. The epitopes/templates were selected due to their negligible natural ability to be imprinted into the PNE matrix and were modified respectively with 3K-tags, in N-, C-, and N/C- positions. The PNE-based MIP developed by exploiting the N-3K tag strategy allowed the development of a sensitive and reproducible SPR biosensor for PD-L1 detection. PD-L1 quantification was accomplished both in buffer and human serum samples ( $LOD = 0.31 \pm 0.04 \text{ ng mL}^{-1}$ ,  $\%CV_{av} = 4.5\%$ ), covering the median human level of this emerging biomarker. This promising strategy opens new insights both for the in-silico rational design of epitopes for pCAs-based mimetics and as a triggering toolbox when native epitopes display negligible imprinting capabilities.

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**GOLD SURFACES FOR BIOSENSOR APPLICATION: BIO-FUNCTIONALIZATION AND ANALYTICAL DETECTION OF VIRAL NUCLEOCAPSID PROTEINS**

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Biosensors based on electrolyte-gated organic field effect transistors (EG-OFET) have been proven capable to selectively detect clinically relevant biomarkers, down to the single-molecule detection limit [1–3], with minimal sample pre-treatment. Both buffered solutions and body fluid have been tested for the assay. Such devices are endowed with high selectivity due to the presence of densely packed layer of biorecognition elements (such as antibodies) on the active gold electrode of the biosensor [4]. One of the simplest methods for the bio-functionalization of the sensor interface is the physisorption of such bio-recognition element directly on bare gold. This strategy holds multiple advantages: it is cost-effective, faster and compatible with printing techniques and requires small amount of reagents. Nevertheless, the physical adsorption reduces the uniformity and the long-term stability of obtained bilayers, as well as the availability of active binding sites. Alternatively, chemical routes can be used to covalently bound the bio-recognition element to the surface, by means of self-assembled monolayers holding the proper functional groups. In this study, the two different bio-modification strategies have been compared in terms of bilayer efficacy towards the detection of nucleocapsid proteins, relevant for the recognition of the HIV virus [5]. Multi-Parameter Surface Plasmon Resonance (MP-SPR) has been used as optical technique to independently characterize the bio-recognition layer and the kinetic parameters of the binding event. The proper immobilization of the antibodies for the recognition of the target protein can be used to guarantee the selective and highly sensitive detection. Relevantly, the same biosensor structure can be optimized for the recognition of different biomarkers, in the attempt of assessing a multiplexing platform for the early recognition of clinically relevant pathogens.

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## GRAPHENE-PAPER ELECTRODES AS NEW PLATFORMS FOR AMPEROMETRIC BIOSENSING

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Graphene and its derivatives are innovative materials widely applied in many fields thanks to their electrical, mechanical, and chemical properties. In particular, graphene paper (G-paper) is a paper-like material possessing high flexibility and large surface area; it can be shaped in different geometries, featuring a high electrical conductivity (*ca.*  $10^5 \text{ Sm}^{-1}$ ), tunable surface chemistry and mechanical stability even after hundreds of thousands bending times [1]. We recently proposed the use of G-paper for the realization of electrochemical sensors (named GPE) on flexible plastic and textile supports (Fig. 1) [2]; they were applied in the detection of lactate in sweat, featuring higher selectivity with respect to commercial, carbon-based screen-printed electrodes (C-SPE and Gr-SPE), thanks to the activation of electrocatalysis towards NADH oxidation. The properties of G-paper can be further tuned by inclusion of various components: the inclusion of graphene oxide (GO) gave better electrocatalytic performance for the electrochemical oxidation of  $\text{H}_2\text{O}_2$ , while functionalization with Prussian blue improved  $\text{H}_2\text{O}_2$  reduction. This allowed to increase the application spectrum of the novel functionalized G-paper platforms, to obtain wearable biosensors for the detection of biomarkers such as glucose, using glucose oxidase as mediator on the electrode surface.

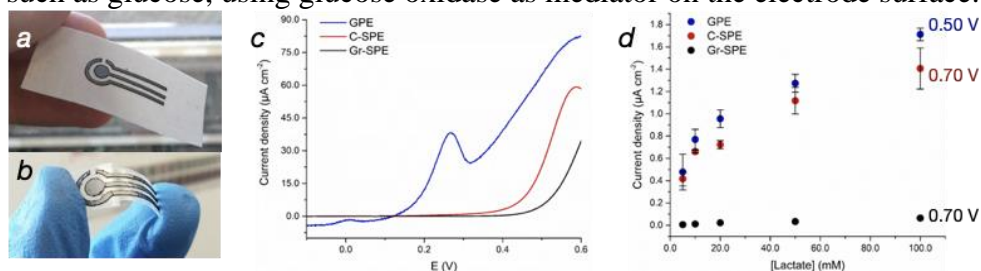


Fig. 1 G-paper based electrodes on *a*) cotton and *b*) PET; *c*) voltammetric signal (blue line) recorded at 1.0 mM NADH (0.1 M PBS, 0.1 M KCl) in comparison to commercial devices (red and black lines); *d*) calibration plot of lactate in sweat.

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**PLASMONIC APTASENSOR WITH FUNCTIONAL ANTIFOULING SURFACE FOR DETECTION OF LYSOZYME IN REAL MATRIX**

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Antifouling coatings are strictly required in optical biosensors for many analytical application fields, from medical diagnostics to foodborne pathogens or antimicrobial agents detection, to prevent the non-specific protein/cell adhesion and select the analytes directly in the complex media [1]. Over the past three decades, the advances in non-fouling plasmonic surfaces have been mainly focused on the detection of ultrasensitive clinical targets in biological samples [1,2], while the development of antifouling coatings for directly analysing complex sample media for food quality control and safety has been scarcely investigated [3]. Herein, we propose a new low-fouling poly-L-lysine (PLL)-based polymer for the detection of lysozyme, an antimicrobial additive with highly allergic risk on consumers and induced amyloid aggregation at high concentration, directly in food matrix by using Surface Plasmon Resonance technology. The PLL-based polymer contains densely immobilized anionic oligopeptide side-chains to create a charge-balanced layer, able to repel the non-specific adsorption of undesired molecules on the biosensor surface. Also, PLL-based polymer includes sparsely attached aptamer probes with high affinity and specificity for lysozyme capturing directly in milk samples. The sensitivity and affinity of lysozyme's aptamer have been deeply explored by changing the ionic strength, ions, and type of buffer, to obtain a label-free protein detection at the nanomolar level directly in food samples. The work here described highlights the versatility of the antifouling aptasensor, which can be applied for the sensitive detection of lysozyme in different complex media, such as food or biological matrices, with minimal pre-analytical sample treatments.

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**IMPLEMENTATION OF EXPERIMENTAL DESIGN TECHNIQUES  
TO OPTIMIZE IMMUNOGLOBULINS DETECTION WITH SIMOA  
SP-X SYSTEM**

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The measurement of ultralow levels of proteins plays a pivotal role in a number of disciplines, particularly in the diagnostic field. Indeed the early diagnosis of diseases through the ultrasensitive detection of protein biomarkers is of paramount importance [1]. In recent years, digital measurement methods such as Single Molecule Arrays (SiMoA) and digital Enzyme-Linked Immunosorbent Assay (ELISA) have made significant progress in terms of ultrasensitive protein detection with different clinically relevant biomarkers [2]. The SiMoA technology, unlike the conventional ELISA, is based on the high-density printing of capturing antibodies in each well of a microtiter plate. Next, the biotinylated detector antibody (in solution) forms a "sandwich" with a target antigen in between. The formation of the immunocomplex is detected through the reduction of H<sub>2</sub>O<sub>2</sub> catalyzed in the presence of a chemiluminescent substrate by Streptavidin Horseradish Peroxidase (SA-HRP) bound to the biotinylated antibody [3]. While printing arrays of antibody has proven to be manufacturable, its uptake has been limited, as it requires highly optimized instrumentation and printing processes. To overcome this issue many laboratories use commercially available pre-rated and validated kits for specific biomarkers. Alternatively, Simoa Planar Array Homebrew kit is commercially available. It can be used to develop custom assays suitable for the immobilization of the desired capture antibody through a pair of peptide tags of an “anchor” antibodies printed on the well surface. A Simoa Planar Array Homebrew Kit for the detection and quantification of IgM, non-specific indicator of inflammation, has been developed and optimized. The experimental design has been undertaken to optimize the assay, leading to reduced experimental effort as well as increased quality of the information obtained with respect to the traditional 'one-variable-at-a-time (OVAT)' approach [4].

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## NEW APPROACHES FOR ELECTROCHEMICAL DEVICES DEVELOPMENT BASED ON CARBONACEOUS MATERIALS INTEGRATING GOLD NANOSTRUCTURES

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In the last decades, advances in nanotechnology dramatically boosted the employment of carbonaceous (nano)materials in the electro-sensing field. In this framework, an appealing research topic regards the integration of noble-metal nanoparticles within conductive carbon-based 'networks', to give rise to effective nanocomposites with superior electro-analytical features.

Herein, two innovative strategies based on carbon surface decoration with gold (Au) nanostructures are proposed.

(i) In the first approach, carbon black (CB), a 0D nanostructured material not normally water 'soluble', was strategically dispersed in water through a sonochemical treatment assisted by rosmarinic acid (RA), an antioxidant phytochemical. The RA-assisted dispersion resulted in colloidal CB nanospheres functionalized with redox moieties derived by the RA catecholic groups, which were strategically exploited to self-assembling Au nanoparticles (NPs) onto the CB surface. CB-RA/AuNPs resulted in enhanced electron kinetics, with superior performance compared to CB dispersed with conventional surfactant and solvents, both in model systems and real analytical applications.

(ii) In the second strategy proposed, a carbon graphite ink has been simultaneously sintered and modified with AuNPs with a laser-based strategy. In brief, Au(III) has been integrated within the ink, the ink was printed and subjected to a CO<sub>2</sub>-laser engraving, which induced the concomitant ink activation and AuNPs formation. The AuNPs-carbon ink was employed to produce stencil-printed electrodes onto PVC supports. The electrode was integrated into a paper-based device, that was applied for the extraction-free determination of the phenolic fraction of different vegetable oils. In this approach, a dedicated paper strip allows the oil sampling and extraction, permitting the transport of the analyte to the electrode surface.

In conclusion, a green approach to obtain 'water-soluble' CB decorated with AuNPs is proposed, and a device able to measure phenols without extraction in vegetable oils has been developed. The proposed strategies provide a sustainable alternative to eliminate the use of solvents and chemicals, and a new approach to develop integrated analytical devices.

**JAGGED1-FLUC: A BIOLUMINESCENT RECOMBINANT PROTEIN AS A NEW TOOL TO IMPROVE THE EARLY DETECTION AND DIAGNOSIS OF COLORECTAL CANCER**

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Colorectal cancer (CRC) ranks as the second among the causes of tumor death worldwide and the discovery of new predictive biomarkers is highly demanded. Recent studies suggest a pivotal role of “abnormal” Notch signaling activation in CRC, partially due to cross-talks of Notch with other pathways such as Wnt and Erk/MAPK. In this context, we developed a recombinant protein using the Baculovirus expression system, which combines the extracellular domain (ECD) of the Notch high affinity mutated form of one of the selective Notch ligands Jagged 1 (Jag1) fused with a Red emitting firefly luciferase, in order to study if Jag1-FLuc binding correlates with a Notch signaling activation in CRC progression. The light emission from the BL cells was evaluated by luminometric assays and imaged by using microscope techniques. Firstly, we set up the optimized conditions for Jag1-FLuc in a cell-free system and then move on to a cell model of human colorectal adenocarcinoma cells (Caco2), which express high levels of the Notch3 isoform. In Caco2 ( $1 \times 10^5$  cells/well) we observed that BL signal increase was proportional to the Notch3 expression, with a linear range from 0.1 to 50  $\mu\text{g/mL}$  of Jag1-FLuc obtaining a LOD and LOQ of  $0.8 \pm 0.2$  and  $6.0 \pm 0.2 \mu\text{g/mL}$ , respectively. In parallel, imaging experiments were performed to examine the light output of the Jag1-FLuc construct on Caco2. We successfully demonstrated the possible application of the bioluminescent Jag1-FLuc protein both for quantification and imaging of Notch expression on Caco2. The bioassay fulfills all the standard criteria of precision and accuracy to ensure the validity and reliability of the method.

Jag1-FLuc represents an important new tool to improve the early detection and diagnosis of pre-neoplastic and neoplastic lesions. The identification and validation of Notch signature, potentially associated to a high risk of tumor progression could suggest a wide range of potential clinically relevant applications including new screening and therapeutic approaches for CRC.

**POINT-OF-CARE DETECTION OF SARS-CoV-2: A NOVEL IMMUNOSENSOR USABLE AT VOLTAMETRIC AND IMPEDIMETRIC TRANSDUCTION MODE**

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COVID-19 has spread globally since its discovery in China in December 2019, causing a growing demand for rapid and accurate diagnostic assays to enable mass screening and testing of both high-risk groups and normal population [1]. According to WHO, the current gold standard for the detection of SARS-CoV-2 is RT-PCR, but has limitations, such as low specificity, long response time, expensive instruments, well-trained personnel and no point-of-care (POC) use [1-3]. Therefore, early, rapid and more sensitive detection methods of SARS-CoV-2 would represent a significant achievement to identify positive patients, allowing crucial decisions and public health strategies by healthcare providers and policy makers.

In this work, a new biosensing platform for the detection of SARS-CoV-2 usable both at voltametric and impedimetric mode is reported. The platform consists of a MWCNTs-screen-printed electrode functionalized with electro-adsorbed methylene blue (MB) and bio-active layers of chitosan/protein A to enhance the bio-activity of the immobilized SARS-CoV-2 antibodies.

Cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) techniques evaluated the fabrication steps of the immunosensors. A change in mass and thickness on the electrode surface will be expected, due to the formation of the antibody-antigen complex, which blocks the electron transfer of the redox probe  $[(\text{Fe}(\text{CN})_6)]^{3-/4-}$  to the electrode surface, causing a decrease of the current signal detected by differential pulse voltammetry (DPV), and an increase of the charge transfer resistance ( $R_{ct}$ ), detected by EIS. A comparison of the analytical performances of the voltametric and impedimetric-based immunosensors is reported. The proposed devices were successively applied to the detection of the SARS-CoV-2 in real saliva samples.

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**HYBRID Au@SiNWs ARRAYS AS POTENTIAL SERS PLATFORMS: SYNTHESIS, ANALYTICAL CHARACTERIZATION AND APPLICATION**

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Surface enhanced Raman spectroscopy (SERS) is widely applied for detection and quantification of target species in early diagnosis [1] or environmental monitoring [2]. It is then highly advisable to develop novel hybrid materials capable to exert controlled enhanced Raman effect coupling plasmonic metal nanoparticles (e.g. Ag or Au NPs) with ordered and dense arrays of Silicon nanowires (SiNWs), as recently reported [3]. Here we report on the modification of SiNWs by electrophoretic deposition (EPD) of chemically produced AuNPs. SiNWs are prepared by a wet-etching technique, assisted by the deposition of an ultrathin metal film on (p-, n-doped or highly doped) Si single crystal [4]. AuNPs are synthesized using stainless steel as solid reductant for HAuCl<sub>4</sub> [5]. Comparison of EPD process with conventional reduction of gold precursors on SiNWs in terms of AuNPs morphology and size, their distribution and density will be offered. The role of silicon doping will be investigated, as well. To this aim, detailed analytical characterization (SEM, TEM, XPS, FTIR, CV) of the proposed materials will be presented. SERS performance will be evaluated on model analytes.

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**SMART AND PORTABLE ELECTROCHEMICAL IMMUNOSENSOR FOR SARS-COV-2 SPIKE PROTEIN DETECTION WITH MACHINE LEARNING FEATURES**

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In the context of the management of the SARS-CoV-2 pandemic, it has become increasingly evident that correct sample classification is critical to containing the virus outbreak. To this end, we developed an IoT-WiFi smart and portable electrochemical immunosensor for the detection of SARS-CoV-2 spike protein integrated with Machine Learning (ML) features. In recent years, the rapid development of ML has highlighted the benefits this technique could offer in integration with biosensors, as new strategies for overcoming signal noise limitations and enabling intelligent predictions for sample classification based on decision-making algorithms [1].

The developed immunosensor is based on the immobilization of monoclonal antibodies directed to SARS-CoV-2 S1 on Screen-Printed Electrodes using an analytical protocol optimized by Full-Factorial Design that involves a single-step one-hour sample incubation. Validation of the immunosensor was performed in viral transfer medium, which is a complex matrix commonly used for desorption of nasopharyngeal swabs, obtaining a limit of detection at low ng/mL level. Furthermore, remarkable specificity was demonstrated by testing viral antigens of influenza A and MERS, which led to obtain responses not significantly different ( $p > 0.05$ ) from negative controls. The immunosensor was interfaced with a WiFi-based portable potentiostat, which is based on a previously developed acquisition device [2,3] now integrating ML features for sensing data analysis. Using different supervised ML algorithms, excellent classification of positive and negative samples was achieved with 97.3% test accuracy. Finally, the immunosensor was successfully tested using a lentiviral vector pseudotyped with SARS-CoV-2 spike glycoprotein, thus proving the applicability of the immunosensor to whole virus detection.

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**ELECTROCHEMICAL LATERAL FLOW IMMUNOASSAY FOR IMPROVED ANALYTICAL PERFORMANCES.**

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The lateral flow immunoassay technique (LFIA), also known as rapid diagnostic test (RDT), has become one of the most successful analytical platforms for decentralized or point-of-need testing strategy requiring little to no supporting infrastructure [1]. The LFIA can be considered as a sort of lab-in-a-hand and together with other point-of-need tests has represented a paradigm shift from sample-to-lab to lab-to-sample aiming to improve decision making and turnaround time. The rapidity, simplicity, relative cost-effectiveness, and the possibility to be used by nonskilled personnel contributed to the wide acceptance of LFIAs.

Although LFIAs are inherently the ideal qualitative screening method, they have been asked to be a quantitative method that allows the analyte ultrasensitive detection. A lot of efforts have been made in this direction thanks to the use of new labels and/or strategies to improve the sensitivity, and to the use of dedicated strip readers. The increasingly pressing demand for the objective quantification and the sensitivity improvement are among the hot challenges that future LFIA devices will have to face with.

Compared with colorimetric, optical, magnetic and other highly sensitive detection methods, the electrochemical detection is well developed with high sensitivity, selectivity and repeatability. Moreover, the increasing compatibility of interfaces with miniature potentiometers can allow electrochemical sensors to become more integrated, automated and intelligent, highlighting their huge potential in future developments. Recently some examples regarding the electrochemical detection coupled to LFIA (eLFIA) have been reported in the literature stimulating research interest [2]. In this communication a comparison between the traditional LFIA and the eLFIA will be presented. We used the detection of the prostate specific antigen as the model system and we exploited different noble metal nanoparticles to investigate the best experimental set up for improved analytical performances.

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**DISPOSABLE LABEL-FREE VOLTAMMETRIC IMMUNOSENSOR FOR SENSITIVE DETECTION OF INTERLEUKIN-6.**

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Interleukin-6 (IL-6) is a soluble mediator with a pleiotropic effect on human health [1]. High concentrations of IL-6 are correlated with a wide range of diseases as well as in the hyper-inflammatory responses, autoimmune processes, and many illnesses such as diabetes, atherosclerosis, depression and Alzheimer's [2-4]. Recently, IL-6 has assumed utmost importance as a poor prognostic factor in patients affected by acute respiratory syndrome due to COVID-19 disease, and its monitoring was crucial to preventing Intensive Care Unit (ICU) demand and improving patient's rate of survival [5].

Herein, the fabrication of a novel label-free voltammetric immunosensor based on biocarbon nanomaterial (biochar) modified screen-printed electrodes (Bio-SPEs) for the rapid, low-cost and accurate detection of IL-6 was presented. A carbodiimide-mediated amide coupling reaction (EDC/NHS) to immobilize the secondary antibody (Ab-IgG) onto biochar's carboxylic group and two different primary receptors, identified as mAb-IL-6 clone-5 and clone-7 antibodies, were used. The electrochemical characterization of the layer-by-layer assembly of the immunosensor was conducted by cyclic voltammetry (CV) and sensing was performed using square wave voltammetry (SWV). The two developed immunosensors, using clones-5 or 7 monoclonal antibodies, respectively, had good analytical performances in human serum, exhibiting a wide linear range (LR) from 12-205 and 15-238 pg/mL, a good limit of detection (LOD) of 8.8 and 10.4 pg/mL and selectivity for IL-6 over other common cytokines, including IL-1 $\beta$  and TNF- $\alpha$ . Performance comparison of IL-6 immunosensors with those of a spectrophotometric ELISA kit (LOD of 20 pg/mL) denoted a better sensitivity of the proposed label-free devices, associated with a reduced detection time (30 minutes instead of more than three hours for ELISA test). Furthermore, the successful application of the proposed immunosensors in blood samples (with only a dilution of 1:100 v/v in PBS and without additional treatments) with good sensitivity (LOD of 14.3 pg/mL and reproducibility, RSD% < 11%) paves the way for their application as a point-



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**A NOVEL INTEGRATION OF MOLECULARLY IMPRINTED POLYMERS WITH NANOPOROUS SILICON FOR SELECTIVE AND SENSITIVE OPTICAL DETECTION OF PROTEINS**

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Molecularly Imprinted Polymers (MIPs) during the last decades have attracted an increasing interest due to their ability of acting as the synthetic alternative to biological receptors, with a higher stability, lower cost, synthesis adaptable to any target analyte, yet keeping high selectivity [1]. For sensors assembly a key issue is MIP integration with the transducer surface, especially for nanostructured materials. To this aim, several strategies have been used [1], including electro- and chemical polymerization, deposition from a pre-polymer solution, and other approaches based on coupling pre-formed MIPs on different surfaces. However, most of the proposed strategies have been optimized on planar surfaces and their application to nanostructured materials is challenging. Thus, a generical approach for the facile integration of MIPs with nanostructured materials that guarantees good adhesion and proper binding sites exposure is of key importance.

In this work, we propose an original approach for MIP deposition on a nanostructured surface, namely nanoporous silicon (PSi) with pores diameter of ~50 nm and high aspect ratio, acting as an interferometer optical transducer. It is based on monomer vapor-phase polymerization upon covalently grafting the template (human hemoglobin) to the PSi surface. Pyrrole has been selected as functional monomer due to the simplicity in achieving thin films from vapor-phase deposition at room temperature, along with its successful use in imprinting procedures. The developed optical sensor exhibited excellent performances in terms of sensitivity, limit of detection, selectivity and application to human plasma and artificial serum. The proposed strategy overcomes limitations restricting MIP integration with nanostructures and can be easily extended to any proteins considering the wide applicability of PPy in the imprinting of macromolecules, and the adopted imprinting protocol.

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**INVESTIGATION OF THE SALT EFFECT AFFECTING THE ACCURACY OF pH COLORIMETRIC SENSOR ARRAYS (CSAs)**

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pH is a key parameter that can be used as a probe for different phenomena (food spoilage, fermentation, ocean acidification, and so on). The glass electrode is traditionally the most important instrument used for the measurement of pH. Nevertheless, it is characterized by various limits such as the signal dependence on the ionic strength due to the variation of the liquid junction potential, the alkaline error, and the slow response in low conductive solutions. In the last years, several efforts have been made to improve the detection of pH. In 2022, the patent “Colorimetric Sensor Arrays (CSAs)” developed in my research group has been successfully granted [1], [2]. The colorimetric sensor is based on a polymer, working as support (for example, PVDF), a gel matrix deposited on the support, and one or two embedded acid-base indicators. A further necessary additive is a suitable charged surfactant. By tailoring its concentration is possible to tune the  $pK_a$  of the indicator to obtain high and homogeneous precision in the entire working interval (0-14). The accuracy is anyway dependent on the saline concentration of the test solution, therefore in this oral presentation, the performance of the CSA will be tested with different solutions characterized by ionic strength changing from 0.01 to 0.40 M. A model including five reactions involving all the components of the CSA will be proposed. The thermodynamic constant describing the equilibrium between the indicator and the surfactant,  $K_s$ , is crucial to determine the dependence of the signal on the ionic strength, and thus the accuracy [3].

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**ELECTROCHEMICAL GATING FOR SENSING: OPERATING PRINCIPLE AND WEARABLE DEVICES**

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In 1984, Wrighton fabricated the first organic electrochemical transistor (OECT) wherein the current flowing between the source and drain terminals was modulated by electrochemical reactions stimulated by the potential applied between the source and gate electrode [1]. He demonstrated the idea of electrochemical gating for controlling charge transport in conducting polymers. Nowadays, OECTs are attracting great attention in chemical sensing due to the intrinsic signal amplification, the high transconductance, the low applied voltages, the biocompatibility and the easy miniaturization. Inspired by OECTs technology, we proposed a new approach for the design of innovative sensors based on conducting polymers functionalized with redox active materials able to exert an electrochemical gating effect that depends on the concentration of target molecules, without the need of an external gate electrical contact [2]. The operation principle of the technology was studied by combining PEDOT:PSS (poly(3,4-ethylenedioxythiophene):poly(styrene sulfonate)) and Ag/AgCl nanoparticles (NPs). In agreement with Nernst law, the Ag/AgCl NPs exert a potential on the conductive polymer whose conductivity is correlated to log [Cl<sup>-</sup>]. pH sensors were obtained by replacing Ag/AgCl NPs with IrOx particles [3] or a layer of PEDOT doped by bromothymol blue [4]. The simplification of the sensor architecture (from 3 to 2 electrical terminals) has been exploited to design and fabricate wearable sensors which are embedded in textile and/or medical dressing able to operate in sweat or wound exudate. The device structure is completed by adding a layer of an adsorbing foam able to generate a bio-fluid flow, which renews the sample close to the sensor.

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**A PAPER ORIGAMI PLATFORM FOR ELECTROCHEMICAL QUALITY CONTROL OF AGRI-FOOD WASTE**

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The over-production of agricultural waste is a global issue that arises from the increasing demand for food. In this context, the *Brassicaceae* family represents a candidate for possible recycling and re-using of the nutritional components [1]. Glucosinolates present in *Brassicaceae* are glucose-containing metabolites involved in the plant defense. When a plant is damaged (e.g., by herbivorous or during crop management), glucosinolates are released in the cytoplasm to react with the myrosinase enzyme [2], producing glucose and by-products [3]. Their loss by volatilization decreases the food composition values [2]. To control the quality of *Brassicaceae* foodstuff and waste, the glucosinolate content needs to be assessed. Besides complex, expensive and reagent/time consuming analytical methods for glucosinolate detection, smarter innovative approaches have been proposed, such as enzyme-based biosensors [3].

In this work, an origami paper-based platform was designed for the evaluation of food quality in *Brassicaceae* samples. A monoenzymatic biosensor and a bienzymatic biosensor were configured together for the detection of glucose and glucosinolates, respectively, using filter paper pads preloaded with glucose oxidase and/or myrosinase enzymes. Office paper-based electrodes modified with Carbon Black/Prussian Blue nanoparticles were employed for the measurement of the enzymatic by-products at a low applied potential (i.e., 0V vs Ag/AgCl). This paper-based platform measured glucose and glucosinolate (i.e., sinigrin) with a linear range up to 2.5 and 1.5 mM, and detection limits of 0.05 and 0.07 mM, respectively. The recovery at two different levels of sinigrin i.e. 0.25 and 0.5 mM assessed values equal to  $(111 \pm 3) \%$  and  $(86 \pm 1) \%$ , respectively. The multiplex detection of glucose and glucosinolate in *Brassicaceae* samples represents a sustainable approach that perfectly matches sustainable agrifood practices with the circular economy vision.

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**SEWAGE SLUDGE-DERIVED BIOCHAR TO DESIGN MORE SUSTAINABLE SENSORS FOR ELECTROANALYTICAL APPLICATIONS**

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In the framework of a circular economy and environmental awareness, the scientific community ought to be continuously on the lookout for more sustainable analytical approaches. The use of recycled carbonaceous materials may represent a promising “greener” alternative to other virgin carbon-based materials in the design of electrochemical (bio)sensors. In this work, we propose the use of biochar, a recycled carbon-rich material derived by controlled pyrolysis or gasification of waste biomass, to develop more eco-friendly carbon paste electrodes. In particular, the carbonaceous paste was composed of 90% biochar obtained by recycling biological sludges originating from municipal and industrial wastewater treatment plants, which usually require expensive disposal processes. The surface of the electrodes obtained was first characterised by analysing the behaviour of common redox indicators and numerous phenols bearing -OH and -OCH<sub>3</sub> moieties in their chemical structure. The information acquired was used to characterise the phenolic fingerprints related to the anthocyanin fraction of three berry fruits, namely *Vaccinium myrtillus*, *Vaccinium uliginosum* subsp. *gaultherioides*, and *Fragaria × ananassa*. Standard anthocyanin mixtures that simulate the natural composition of such berries, as well as fruit extracts, were evaluated and compared by a rapid electrochemical screening. This work supports the applicability of biochar-based electrodes to the electroanalysis of real agri-food samples.

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**DISSIPATIVE DNA NANOTECHNOLOGY FOR ANALYTICAL CHEMISTRY**

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Supramolecular chemistry focuses on molecular assemblies, complex materials and molecular machines built upon intermolecular non-covalent interactions. Supramolecular chemistry is a highly interdisciplinary research area that spans the fields of chemistry, biology, nanotechnology, materials science, and physics. In particular, thanks to the advancements reported in the development of responsive self-assembling systems and molecular machines and in the study and use of synthetic receptors, supramolecular chemistry has been making a significant impact on the development of analytical sciences. The integration of analytical and supramolecular chemistry has promoted the birth of the research area of “supramolecular analytical chemistry”, first termed by Prof. Eric Anslyn [1]. In his original definition, the field involves analytical chemistry applications of synthetic chemical structures and molecular machines that undergo molecular recognition and self-assembly. In the last decade our group has employed synthetic DNA to develop a variety of nature-inspired molecular devices that can find applications in sensing. Using rational design and taking advantage of the programmability of DNA-DNA interactions we have characterized DNA-based devices, machines and self-assembling structures that can be used as electrochemical and optical sensors for multiple targets (proteins, antibodies, pH, small molecules, etc.). Recently we have re-engineered these DNA-based devices so that they can be controlled through dissipative out-of-equilibrium mechanisms. Such dissipative strategies allow to control in an exquisite way fundamental properties of biological systems like adaptability, directed motion, regulation or self-organization. By exploiting dissipative mechanisms in DNA-based systems we provide new properties that can have important analytical applications. For example, dissipative systems show analyte concentration-dependent adaptability achieving real-time quantitative information and reversible multicomponent analysis. Also, the transient response characteristic of dissipative systems can allow to introduce temporal control over the signalling of the responsive devices.

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**POTENTIOMETRY UNRAVELS THE REACTION MECHANISM IN SEMICONDUCTOR PHOTOCATALYSIS**

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Since the pioneering applications of photocatalysis in the fields of energy and environmental remediation were proposed in the 1970s and 1980s, many aspects of the photocatalytic process were thoroughly investigated, and several mechanistic studies tried to justify the peculiar behaviour of the photocatalytic rate. [1] In particular, the basic model proposed by Minero [2] explained the overall photocatalytic process in terms of basic photocatalytic reactions. Following this model, the photocatalytic rate in the absence of back reactions is proportional to the hole concentration in the photocatalyst particles and surface concentrations of reactants. However, the photocatalytic rate could also be measured from the photoelectron density, which can be assessed from the *i*-*V* plot of a semiconducting electrode, monitoring its open circuit potential. [3] We propose that open circuit potential could be used to instantaneously evaluate the photocatalytic rate. We demonstrated that for substrates for which back-reactions can be ruled, such as formic acid and alcohols like methanol and glycerol, the photoelectron density directly gives the photocatalytic rate, which linearly increases with the square root of the substrate concentration as predicted by the basic kinetic model. Moreover, we demonstrated how open circuit potential responds to variations in the incident irradiation in terms of wavelength and photon flux, type and concentration of oxidant and substrate. Compared with the conventional measurement of substrate disappearance, the method here proposed is simpler and significantly faster, allowing the assessment of the photocatalytic rate in the timescales of minutes and hours, when traditional measurement would require considerably longer times, involve trained laboratory staff and dedicated analytical equipment. Conversely, when the substrate is prone to back reactions, e.g. phenol and catechol, we observed a more complex behaviour. In these cases the dependence on the square root of the substrate concentration is not followed, and the photocatalytic rate is a complex function of the photoelectron density. Measurements with phenol or catechol as hole scavengers and with NaIO<sub>4</sub> as an electron scavenger confirmed the relevance of back reactions in the overall photocatalytic process.

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**A PRINTED MICROFLUIDIC WEARABLE SENSOR FOR CORTISOL MONITORING IN SWEAT**

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Monitoring biomarker levels in sweat samples are becoming increasingly relevant as it can provide real-time information about human performance and well-being. Cardiovascular disease (CVD) is one of the main causes of death worldwide, according to the World Health Organization (WHO), and the mortality rate from CVD has increased annually more than any other cause in both developed and developing countries [1]. CVDs can be caused by a range of factors and disorders including high blood pressure, cholesterol, diabetes, obesity and overweight, smoking, and stress. Psychosocial stress is said to be a key cause of CVD among these variables [2]. Cortisol, commonly known as the stress hormone, is a significant component in the psychobiology of the stress response and its detrimental health consequences. It regulates a variety of physiological processes and is crucial for maintaining cardiovascular, immunological, renal, skeletal, and endocrine system homeostasis as well as blood pressure regulation.

Herein, we describe the development of a magnetic beads-based wearable immunosensor for real-time monitoring of cortisol in sweat. The device consists of low-cost fabricated screen-printed electrodes (SPE), fabricated on a polyester substrate, for the amperometric detection of the analyte combined with the microfluidic platform including a paper-based pad in which all the reagents needed to carry on the measurement were pre-loaded. The magnetic beads (MBs) are used as support for the immunological chain. The MBs-based immunosensor was conceived as a direct competitive immunoassay using acetylcholinesterase-cortisol conjugate measuring the enzymatic by-product thiocholine at the screen-printed electrode modified with carbon black/Prussian blue (CB/PB-SPE). MBs were incubated for 1 hour with anti-cortisol monoclonal antibody designed to selectively bind cortisol. Then CB/PB-SPEs were modified with 5  $\mu$ L-drop of anti-mouse antibody MBs. The microfluidic paper-based devices were pre-loaded with 20  $\mu$ L of a solution containing enzyme-labeled cortisol (fixed amount) and cortisol (increasing concentrations), 20  $\mu$ L of acetylthiocholine 10 mM, and let to dry. Finally, amperometric detection was carried out by resuspending all the

reagents with 20  $\mu\text{L}$  of phosphate buffer and applying a potential of 0.5 V for 130 seconds. The linearity was observed in the range comprised between 5 to 140 ng/mL of cortisol described by the following equation  $y = (0.216 \pm 0.004)x + (-0.0005 \pm 0.0001)$ ,  $R^2 = 0.885$ . To evaluate the suitability of the developed biosensor, a sample of sweat collected during running activity was measured, obtaining 76.9 ng/ml, in agreement with physiological values of cortisol in human sweat. The measurements were performed with an NFC-based potentiostat developed by the research group of Prof. Marrocco.

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## LAYERED DOUBLE HYDROXIDES FOR ELECTROANALYTICAL APPLICATIONS

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Layered double hydroxides (LDHs) are synthetic compounds deriving from the naturally occurring mineral hydrotalcite,  $Mg_6Al_2(OH)_6CO_3 \cdot 4H_2O$ , and, consequently, LDHs are also known as hydrotalcite-like materials. There are a lot of combinations of bivalent and trivalent cations that can give rise to LDHs. Their chemical tunability together with large surface area, anions exchange ability and the electrocatalytic behavior of LDHs containing redox active metals are the key features that allow to improve the performances of electrochemical sensors. Moreover, the layered structure can host active molecules, such as enzymes, for the selective sensing based on a bio-recognition element. LDHs can be synthesized by various chemical methods but electrochemical deposition is more suitable to produce thin LDHs films well adherent to the electrode surface.

This contribution summarizes our work on LDHs based sensors (2000-now) focusing on the last advancements.

Firstly, the properties of anionic exchange of LDHs were exploited to develop potentiometric sensors for the detection of chloride, sulfate, and phosphate. Later, Ni and Co based LDHs were used as electrode modifiers for the development of amperometric sensors to detect a lot of oxidizable analytes [1]. Very recently, our research has addressed to improve LDHs electrical conductivity and charge transfer capacity by synthesizing composites based on a Ni-Al LDH and carbon nanomaterials, and to get an amplified signal in respect to the one of an amperometric sensor using the architecture of an organic electrochemical transistor (OECT). In the former case the composites have been tested for glucose electrooxidation, which was exploited for the fabrication of a sensor with high sensitivity ( $2.6 \text{ A M}^{-1} \text{ cm}^{-2}$ ) and low limit of detection ( $0.6 \text{ }\mu\text{M}$ ) [2]. In the latter case the developed biosensors were based on an electrode modified with a Ni/Al LDH entrapping glucose oxidase or lactate oxidase [3] and displayed a current amplified ( $\sim 400$  times) by the transistor architecture for glucose and lactate determination [4].

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**RATIONAL DESIGN OF HAIRPIN DNA REPORTERS FOR ENHANCED CRISPR-BASED DETECTION OF VIRUSES AND BACTERIA**

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Beyond the remarkable editing ability of CRISPR-Cas (Clustered Regularly Interspaced Short Palindromic Repeat-CRISPR-associated) systems, the RNA programmed non-specific (*trans*-) nuclease activity of CRISPR-Cas Type V and VI systems has opened a new era in the field of nucleic acid-based diagnostics [1, 2].

However, when using Cas enzymes without pre-amplification of the DNA or RNA target, most CRISPR-based platforms shows relatively slow signal generation and limited sensitivity. Here, we report on the enhancement of *trans*-cleavage activity of Cas12a enzymes using hairpin DNA sequences as FRET-based reporters. We discover faster rate of *trans*-cleavage activity and improved affinity (Km) of Cas12a for hairpin DNA structures and provide mechanistic insights of our findings through Molecular Dynamics simulations. Through the rational design of hairpin DNA probes, we significantly enhance FRET-based signal transduction compared to the widely used linear single stranded DNA reporters. Our signal transduction enables faster detection of viral and bacterial targets with improved sensitivity and specificity, either in the presence or in the absence of an upstream pre-amplification step.

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**FAST UNTARGETED LIPIDOMICS BY ULTRA-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY-TRAPPED ION MOBILITY MASS SPECTROMETRY: A PROOF OF CONCEPT**

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In the field of omic sciences, lipidomics has emerged as essential approach to shed light on the role of lipids in key diseases and biological processes. Untargeted strategies should keep an adequate balance between throughput and coverage, to be applied in the screening of hundreds of samples and rapidly deliver quali-quantitative data. Trapped ion mobility mass spectrometry (TIMS-MS) has recently showed its potential as additional dimension in hyphenated methods [1]. In this study we developed, validated and applied a fast reversed phase ultra-high performance liquid chromatography-trapped ion mobility mass spectrometry (RP-UHPLC-TIMS-MS) method for untargeted lipidomics. A short, narrow-bore fully porous particle charged surface hybrid (CSH) column (50 mm × 2.1 mm, 1.7µm, 130Å) was used, and by selecting appropriate flow rate, temperature and gradient conditions, the total analysis time was scaled from 20 to 4 min. TIMS was operated in parallel accumulation serial fragmentation mode (PASEF) which allowed to select multiple precursors for MS/MS and separate co-eluting lipids based on their different mobility. Lipid annotation was performed by rule-based approach, comparison with LipidBlast spectral library and manual data curation, by taking into account class-specific fragmentation pattern, accurate mass, adduct form, retention behavior in RP and comparison of their collision cross-section (CCS) values for increased confidence. 306 unique lipids from 21 subclasses were annotated from 20 µL of plasma, while their concentration was estimated by class-specific deuterated internal standards. The analytical method was validated and finally applied to elucidate the alteration of plasma lipid profiles in a small cohort of amyotrophic lateral sclerosis (ALS) patients. Univariate and multivariate statistics evidenced significant differences with respect to control patients, particularly in the levels of ether linked lipids (PC-O, PE-O, PE-P and LPC-O), sphingolipids (Ceramide), and triacylglycerols. These results show the usefulness of this fast approach in providing accurate and rapid results with respect to longer (≥ 15 min) untargeted UHPLC-HRMS methods.

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**HUMAN CHEMOSIGNALS ELICITED FROM EMOTIONAL STIMULATION: AN INVESTIGATION BY COMPREHENSIVE GCXGC HIGH RESOLUTION MS**

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Tough the existence of human chemosignals has never been demonstrated, body odour is known to affect the emotional state of a receiver [1]. Within the Potion project, we investigated compositional changes of sweat volatiles in 40 volunteers undergoing fear stimulation in highly controlled conditions. Chemicals were collected by pre-treated pads and then extracted, enriched, and trapped into Tenax GR tubes with dynamic headspace (DHS at 60°C). Analyses were carried out by comprehensive two-dimensional gas chromatography (GCxGC) and time-of-flight mass spectrometry (TOF), whereas emotions were induced by immersing individuals wearing mobile sensors (EDA, ECG, respiratory rate) in virtual reality scenarios.

Emotional rankings showed the successful induction of fear and significant differences in sensor features, in accordance with literature. We tentatively identified 311 compounds, assigned 38 class unknowns, and can report 15 true unknowns. This set of 364 compounds was monitored with high selectivity across samples. We further narrowed down the detected volatiles to a set of 287 sweat volatiles with significantly different concentration values compared to field blanks (fold change FC < 3.0,  $p_{\text{FDR}} < 0.05$ ). Our method shows a good compound coverage with 581 known skin volatiles [2], and a perfect match for 116 compounds. Possible limitations are the detection of certain organic acids and a few very volatile compounds. Out of these compounds, we can report a subset of 24 sweat volatiles that were significantly increased during fear vs. relaxed condition (FC:  $2.21 \pm 0.78$ ,  $p_{\text{FDR,BH}} < 0.05$ , with paired t-tests). Only five of these compounds have previously been reported as skin volatiles and only half of them have so far been detected in humans.

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**DEVELOPMENT OF A RPWAX×RPLC-HRMS PLATFORM FOR THE NON-TARGETED IDENTIFICATION OF PFASs IN AQUEOUS FILM-FORMING FOAMS.**

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The growing interest in two-dimensional liquid chromatography (2D-LC) is due to the significant increase in peak capacity that can be achieved by the combination of distinct chromatographic selectivities to extend the knowledge on the composition of complex samples [1], as demonstrated for synthetic and natural polymer blends, natural compounds, and biomolecules [2]. This research aims at the development of a novel 2D-LC method coupled to high-resolution mass spectrometry (HRMS) for the identification of anionic and zwitterionic poly- and perfluorinated compounds (PFASs) in fire-fighting aqueous-film forming foams (AFFFs), laying the groundwork for the analysis of these compounds in environmental matrices. In detail, the 2D-LC-HRMS method was developed by investigating the combination of mixed-mode weak anion exchange stationary phases with the traditional octadecyl reversed-phase, to obtain a separation in the two-dimensional space according to the anionic classes of PFAS and their chain length. The orthogonality studies and the optimization of the two-dimensional configuration were performed on a training set of twenty-four anionic PFASs, representing the main classes of compounds occurring in AFFFs and covering a wide range of physicochemical properties. The 2D-LC method development was carried out investigating full gradient setups in combination with active solvent and stationary phase assisted modulations. Furthermore, the 2D-LC-HRMS data from method development and sample analysis were processed using an on-purpose algorithm based on the Self-Adjusting Feature Detection [3], here implemented for the non-targeted feature detection in the two-dimensional space. Finally, the developed method was applied to the non-targeted analysis of two AFFF samples for the identification of homologue series of PFASs.

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**TARGETED AND UNTARGETED CHARACTERIZATION OF UNDERIVATIZED POLICOSANOLS IN HEMP INFLORESCENCE BY LIQUID CHROMATOGRAPHY-HIGH RESOLUTION MASS SPECTROMETRY**S. E. Aita<sup>1</sup>, A. L. Capriotti<sup>1</sup>, A. Laganà<sup>1</sup>, L. Mondello<sup>2</sup><sup>1</sup>Dipartimento di Chimica, Università di Roma “La Sapienza”, Piazzale Aldo Moro, 5 - 00185 Roma<sup>2</sup>Dipartimento di Scienze Chimiche, Biologiche, Farmaceutiche ed Ambientali, Università di Messina, Polo Annunziata - 98168 Messina

Policosanols are long chain aliphatic alcohols, with carbon chains typically in the range 20–36, with interesting biological activities. As such, policosanols find application in developing dietary food supplements and additives, animal feed additives, cosmetics, and pharmaceuticals [1]. The common employed approach for the qualitative and quantitative analysis of policosanols is based on a targeted approach involving separation by gas-chromatography after silylation with mass spectrometry (MS) or flame ionization detector. Only few works described the separation of this class of compounds by liquid chromatography.

The work describes the development of an analytical method based on a targeted and untargeted approach to characterize underivatized wax policosanols in hemp inflorescence [2]. Policosanols can be obtained as a by-product of cannabidiol extraction and were prepared by extraction of the free policosanol fraction and hydrolysis of the esterified policosanols in wax. The samples were analyzed by UHPLC-HRMS with ionization assistance by lithium ion adduct formation. In this way, policosanols could be readily separated under reversed-phase conditions and detected by MS. The MS2 level at low NCE did show a typical profile, with the presence of the lithiated adducts of native policosanols and their water adducts. The procedure was optimized, and a quantitative method was validated for the most abundant policosanols (with C24, C26, C27, C28, and C30 chain lengths) in industrial hemp inflorescence extracts. The method was used for the quantitative analysis of policosanols in five hemp types. Hemp wax was found rich in these compounds, mainly C26 and C28 policosanols, which may prove useful for revalorization of wax by-products. Finally, the acquired data were also used to expand the search to the untargeted qualitative analysis of policosanols using Compound Discoverer. The untargeted method allowed the annotation of underivatized policosanols up to C33. The described method represents a promising starting point for the development of analytical methods for underivatized policosanols also in different matrices

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**COUPLING OF LIQUID CHROMATOGRAPHY TO NEGATIVE CHEMICAL IONIZATION MASS SPECTROMETRY: A NEW ANALYTICAL APPROACH FOR THE ANALYSIS OF PER- AND POLYFLUOROALKYL SUBSTANCES**

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Per- and polyfluoroalkyl substances (PFAS) are a class of aliphatic manufactured compounds comprising fluoro-chemicals with varied functional groups and stable carbon-fluorine bonds. Thermal and chemical stability and simultaneously lipophobic and hydrophobic properties characterize PFAS, supporting their role as additives in several materials [1]. The strong protein affinity makes them highly bio-accumulative and persistent. Their presence has been detected in different environmental samples ranging from water, air, and soil to human blood and demonstrate severe health hazards [2]. High-performance liquid chromatography coupled to electrospray ionization-mass spectrometry is usually applied to analyze the PFAS. Negative chemical ionization (NCI) is generally coupled to GC and provides high selectivity and sensitivity for compounds that contain electronegative atoms, such as PFAS. One of the major applications of NCI-MS has been in screening environmental substrates for the analysis of toxic substances [3].

The liquid electron ionization (LEI) interface is an efficient mechanism developed to couple robustly a liquid flow rate from an LC system to an EI source, by converting the LC elute in the gas phase before entering the EI source [4]. LC-LEI-MS was recently and successfully used in NCI mode for pesticides determination [5]. Considering these results, here we present an analytical method based on LC-LEI-MS in NCI mode for determination of PFAS. The proposed approach aims to combine the chromatographic separation with an ionization mechanism particularly suitable for these target compounds to exploit the selectivity of NCI towards PFAS and increase the method sensitivity. Several columns will be tested operating at different flow rates. A passive flow splitter will allow a lower flow rate (maximum 500 nL/min) reaching the CI source, to maintain the proper vacuum condition needed for the expected ionization efficiency. Quantitative analysis will be carried out on different types of samples.

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**IDENTIFICATION OF PHOTODEGRADATION PRODUCTS OF ESCITALOPRAM IN SURFACE WATER BY HPLC-MS/MS**

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In recent years, the use of antidepressants has grown enormously, and selective serotonin reuptake inhibitors (SSRIs) are the most prescribed. Among these, escitalopram is the most selective and its release into the environment is of concern. Considering the parameters of persistence, bioaccumulation and toxicity, it is classified as high-risk compound [1]. On the other hand, it would be appropriate to know the transformation products (TPs) that naturally can be originated in the environment, assessing their potential dangerousness and developing methods for their determination in water.

This study consists in the evaluation of the photodegradation of escitalopram in model solutions of ultrapure water alone and at different concentrations of nitrate (1-20 mg/L) and bicarbonate ions (1-100 mg/L), and in surface water using solar radiation simulated with a Solarbox.

In order to evaluate the degradation kinetics and the formation of photodegradation products, a rapid liquid chromatography coupled with tandem mass spectrometry (HPLC-MS/MS) method has been developed and validated, particularly optimizing the collision energies of the transitions of the built SRM method, and specifying a dwell time in accordance with the viewing window of the chromatographic peak centred on its retention time. Through experimental design, the effect of the concentrations of nitrate and bicarbonate ions on the formation of photodegradation products was investigated, comparing the obtained values of the photodegradation kinetics. The kinetics are all of the pseudo-first-order ranging from  $t_{1/2} = 2.8 (\pm 0.1)$  days in ultrapure water to  $1.5 (\pm 0.1)$  days in the presence of nitrate and bicarbonate at their maxima investigated concentrations.

Fifteen photodegradation products were identified, their chemical structures were proposed on the basis of interpretation of the obtained MS/MS spectra, and a photodegradation pathway was proposed.

Furthermore, through *in silico* methods, it was possible to verify the TP's toxicity, which is similar to or lower than that of escitalopram.

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**EXPLORING THE RETENTION BEHAVIOR OF CANNABINOIDS UNDER NORMAL- AND REVERSED-PHASE CONDITIONS ON IMMOBILIZED POLYSACCHARIDE-BASED CHIRAL STATIONARY PHASES**

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The popularity and the recent legalization of cannabis products have contributed to the increase of the demand for accurate analytical methods able to provide a detailed characterization of cannabis samples and extracts. In this context, one of the emerging aspects is about the hazardous potential of uncharacterized minor cannabinoids, including chiral ones, for which achiral potency testing methods currently employed do not give any information. For this reason, the development of liquid chromatographic methods for the enantioseparation of cannabinoids is highly necessary [1,2].

This work focuses on the investigation and comparison of the chromatographic behavior of some of the major cannabinoids (including the cannabichromene racemate, CBC) on differently substituted polysaccharide-based chiral stationary phases (CSPs), under both normal- and reversed-phase conditions. The influence on retention of both electron withdrawing or electron donating groups on the CSPs and the chemical structures of cannabinoids has been evaluated.

Results suggest that polysaccharide-based columns are suitable for the analytical separation and quantification of the main cannabinoids present in real samples and that they can be efficiently employed for the characterization of cannabis products and derivatives also from the chiral point of view.

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**DEVELOPMENT AND VALIDATION OF A LIQUID CHROMATOGRAPHY – MASS SPECTROMETRY METHOD FOR MULTIRESIDUE ANALYSIS IN MUSSELS OF THE ADRIATIC SEA**

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The ongoing release of hazardous chemical compounds into the aquatic environment is an issue that has been of increasing concern to regulators. Assessment of aquatic pollution levels is not easy and cannot be based solely on quantification of abiotic components but relies on analysis of the bioaccumulation of xenobiotics in organisms inhabiting the environment, like bivalves, that act as sentinels of the presence and effect of anthropogenic pollutants in the sea water.

Herein we present an optimized and validated method for the determination of pharmaceutical and pesticides residues in mussels (*mytilus galloprovincialis*). Pharmaceuticals and pesticides included in this study were selected based on their large worldwide use, of their legal status and/or their known persistency. A novel sample extraction strategy was optimized by a systematic investigation of the various methods reported so far [1]. Different extraction solvents (hexane, cyclohexane, dichloromethane, acetonitrile, methanol and acetone) as well as different clean-up approaches (Lichrolut and C18 SPE, dSPE, liquid–liquid extraction, CHAPS) were tested.

The optimized method consisted of a double solid-liquid extraction with two solvent mixtures of decreasing polarity followed by the addition of CHAPS as emulsifier and Methanol. After the extraction, samples underwent high performance RP liquid chromatography–triple quadrupole mass spectrometry (LC–MS/MS) analysis in positive mode for the identification and MRM quantitation of the target analytes by using isotopically labelled IS and a matrix-matched calibration approach. The fully validated method was applied on real mussel samples collected in several areas of the Adriatic Sea in different seasons, and the results analysed by univariate and multivariate statistic approaches.

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**DEVELOPMENT AND VALIDATION OF MULTI-CLASSES OF PESTICIDES IN CORN-BASED FOOD USING COMPREHENSIVE TWO-DIMENSIONAL LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY (LC×LC-MS/MS)**

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It is notorious that fruits and vegetables are fundamental for a healthy lifestyle. In fact, the World Health Organization (WHO) recommends the consumption of more than 400 g of vegetable and fruits per day with a low sodium, fat and sugars in order to reduce noncommunicable diseases (NCDs). On the other hand, in modern agriculture, pesticides are practically used all over the year's production. Since they are dangerous to human health, it is important to check pesticide residues after their application to food, thus providing a measure of food quality.

Conventional liquid chromatography do not always provide the resolving power and selectivity needed for the analysis of complex matrices. As a consequence, multidimensional and, especially, “comprehensive” two-dimensional liquid chromatography (LC×LC) methods may be a valuable tool due to better resolving power, peak capacity and decreasing matrix effects.

In this contribution, LC×LC-MS/MS were used for the characterization of multi-classes of pesticides from various corn-based food. In additional, two different extraction method were compared: the most common extraction procedures “QuEChERS” and non-buffered extraction.

Matrix-matched calibration curves were carried out for quantification of all the samples. The figures of merit determined were recovery, linearity, limits of detection and quantification (LoDs and LoQs), precision in term of intra-inter days.

To the best of our knowledge, no previous LC×LC-MS/MS study has been reported with this set-up.

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**SUSPECT SCREENING APPROACH FOR THE IDENTIFICATION OF SULFONAMIDES TRANSFORMATION PRODUCTS IN WATER SAMPLES**

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In the recent years, contaminants of emerging concern (CECs) in the aquatic environment have become an important issue [1]. These compounds mainly come from the effluents of the wastewater treatment plants (WWTP), which are unable to completely remove CECs and their transformation products (TPs). Due to the abuse and misuse, antibiotics are one of the main pharmaceutical groups among CECs and are responsible for the emergency of antibiotic resistance. Sulfonamides (SAs) are among the most widely used antibiotics for both humans and animals, and many studies have reported their presence in surface water; furthermore, their TPs are of great concern, since they could still exert some pharmaceutical activity. The transformation processes can be accomplished by biological metabolism, WWTP activities, environmental and other abiotic and biotic factors; a comprehensive view of all the possible transformation pathways is challenging, since many TPs are still unknown. This lack of knowledge makes the monitoring of SAs and their TPs a major analytical challenge hampered by the complexity of the matrices, the huge number of different compounds, and their low concentration levels. In the present study, an analytical approach for the preconcentration and identification of SAs' TPs has been developed. First, a solid phase extraction protocol using Graphitized Carbon Black sorbent was set up and compared to a literature protocol using the popular Oasis HLB sorbent. Later, three of the most used SAs (sulfamethoxazole, sulfapyridine, sulfadiazine) were incubated for 20 days in a batch reactor with activated sludge under controlled conditions. The obtained TPs were then tentatively identified by an innovative data treatment workflow on Compound Discoverer based on the *expected compounds* approach and manual validation of the fragmentation spectra. The list of the 44 annotated TPs was then employed as an inclusion list for subsequent suspect screening analysis of four WWTP samples from different areas of Milan.

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**EFFECTS OF MULTIFUNCTIONAL BUFFERING AGENTS AND ADDITIVES OF THE BACKGROUND ELECTROLYTE SOLUTION ON ELECTROOSMOTIC FLOW AND MIGRATION BEHAVIOUR OF THE ANALYTES IN CAPILLARY ZONE ELECTROPHORESIS**

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This communication discusses the use of buffering agents and additives of the background electrolyte solution (BGE) tailored for capillary zone electrophoresis (CZE) of biomolecules bearing different concomitant functionalities, such as ionizable and/or hydrogen-bonding groups, hydrophobic regions, and hydrophilic moieties. Most of these compounds are expected to interact to different extents with the components of the BGE and with the inner surface of the capillary tube, with consequent poor efficiency, asymmetric peaks and other untoward phenomena.

Most of the buffering agents and additives employed to overcome such drawbacks discussed in this presentation include aliphatic vicinal oligoamines, which, in combination with polyprotic acids, are effective at controlling the protonic equilibrium in a wide pH range and at preventing or minimizing untoward interactions between the above analytes and the capillary wall. Other investigated additives function as strong ion-pairing agents or competing compounds for the interacting moieties of the analytes exposed to the BGE, subtracting their availability to the interacting sites on the capillary wall. Some other, such as 2,2,2-trifluoroethanol, have a strong impact on the electrophoretic behavior of the analytes, influencing both their electrophoretic mobility and the generation of the electric double layer at the interface between the inner surface of fused silica capillaries and the electrolyte solution, which originates the electroosmotic flow.

Also illustrated and discussed is the employment of strong alkaline electrolyte solutions for the separation of carbohydrates at pH values at which they are totally ionized (pH 12-13) and, therefore, separated as anions. The direct detection by UV of the separated carbohydrates is carried out at 270 nm, due to a base-catalyzed conversion into UV absorbing substances, initiated by the light of the UV detector lamp while the analytes are passing the detection window of the capillary tube, due to a base-catalyzed conversion into UV absorbing substances of the separated carbohydrates.

Practical applications of the investigated buffering agents and additives of the BGE to the development of CZE methods for the analysis of biomolecules in complex matrices are reported. The discussed applications will further demonstrate that selecting proper pH and composition of the electrolyte solution is of paramount importance in optimizing the separation of complex biomolecules by CZE.

**THE COMBINATION OF RPLC-ESI-FTMS/MS AND *m*-CPBA EPOXIDATION FOR DOUBLE BOND LOCATION AND GEOMETRY ASSIGNMENT IN UNSATURATED FREE AND TOTAL FATTY ACIDS**

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Linseed, poppy seed, and walnut oils have been largely employed as effective binders or paint media named drying oils to grip pigments onto the surface of artwork supports. Being rich in triacylglycerols and bearing high amounts of (poly)unsaturated fatty acyl ((P)UFA) chains, the broad use of such oils paved the way for a novel artistical technique called *oil painting* [1]. The interaction between drying oils and atmospheric oxygen leads to the conversion of a drying oil into a densely cross-linked polymer network through radical reactions [2]. As the drying oil ages, some degradation phenomena may be triggered resulting in protrusions and efflorescence on the artwork, named metal soaps [3]. Since both the curing and degradation processes strongly depend on the fatty acyl chain profile of the oil, many studies have been focused on the fatty acidome of drying oils. Despite providing no hints about the FA occurring as a metal soap, both Fourier-transform infrared spectroscopy (FTIR) and X-ray powder diffraction (XRPD) are the most common less invasive techniques for the diagnosis of metal soap formation. For this purpose, much benefit may derive from the investigation of drying oils via mass spectrometry (MS), which represents a suitable strategy to address the fatty acidome of cultural heritage specimens [4]. Therefore, both the free (FFA) and total fatty acids (TFA) of mostly used drying oils were investigated by reversed-phase liquid chromatography coupled with high-resolution mass spectrometry by an electrospray ionization source (RPLC-ESI-FTMS) [5]. Here, carbon-carbon double bonds of UFA were derivatized into epoxides [epoFA] by *meta*-chloroperoxybenzoic acid (*m*-CPBA) and subjected to tandem MS to assign the double bond localization [6], whereas the retention times of epoFA provided evidence on their double bond *cis/trans* geometry [7].

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**AMBIENT MASS SPECTROMETRY AND SWABBING TEST STRATEGIES FOR INNOVATIVE HIGH-THROUGHPUT ANALYSIS OF ALLERGENIC PROTEINS**

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Careful allergen management through a reliable risk assessment of potential cross-contaminations is required to prevent allergic reactions in sensitised individuals. Food producers, restaurants and canteens have to implement proper cleaning procedures on production lines and food preparation surfaces to ensure proper protein removal. The validation and continuous monitoring of the cleaning process imply sampling strategies coupled to screening analytical methods [1].

Ambient mass spectrometry (AMS) has grown into a group of emerging analytical techniques which combine the inherent selectivity and sensitivity of mass spectrometry with the possibility to perform rapid, real-time, high-throughput, in situ analysis, with minimal or no sample pretreatment. These features map well against the Green Analytical Chemistry principles.

In this context, for the first time, the present research project aims at (i) investigating the potential of different ambient ionization MS techniques to analyze allergenic food proteins as well as (ii) developing a suitable swabbing strategy compatible with the philosophy of ambient MS methods, i.e. minimal and fast sample handling, for allergen residue analysis.

For these purposes, paper spray ionization, desorption electrospray ionization as well as swab touch spray ionization are explored to detect both intact allergen protein and corresponding tryptic peptides. Critical parameters and key experimental variables of each technique were investigated in terms of their effect on desorption and ionization efficiency. In addition, strategies for in situ and fast extraction, enzyme digestion and swabbing sampling were devised to expand applicability of AMS to safety concerns related to food allergens.

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## HIGH-RESOLUTION MS DETECTION OF PESTICIDES USING THE DIRECT COUPLING OF A SPME FIBER WITH A LIQUID-EI INTERFACE

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Since the first introduction, thousands of papers on solid-phase microextraction (SPME) have been published, demonstrating the worldwide success of the technique with both GC- and LC-MS instruments [1]. Recently, a novel microfluidic technology, called microfluidic open interface (MOI) was developed for the direct coupling of SPME fibers to a liquid electron ionization-tandem mass spectrometry system (LEI-MS/MS) [2]. Because the LEI interface is coupled with a conventional EI-based MS, it allows operation with different ionization modes, such as EI or chemical ionization (CI) both in positive and negative ion modes. Negative chemical ionization (NCI) MS and MS/MS allow to achieve high selectivity and sensitivity for electrophilic compounds in complex matrices, minimizing the chromatogram background noise, reaching high signal-to-noise ratio and ppt detection limits [3]. In this work, a modified MOI equipped with a passive flow splitter (PFS) was coupled with LEI-QTOF, in NCI mode. Dicamba and tefluthrin were used as model compounds in a commercial formulation and water. The analytical procedure involves the direct immersion of a C18 Bio-SPME fiber in 1 ml of sample, fortified with the model compounds, for 3 minutes. The fiber desorption was performed by plunging the fiber in 2.5  $\mu$ L of acetonitrile inside the MOI chamber. The flow rate was set at 10  $\mu$ L/min to allow a rapid transfer of the desorption solvent, splitted at a ratio of 1:20 before LEI, to meet the required nano-flow rate. The experiments were conducted in scan mode and the matrix effects were evaluated by comparing the calibration curves of the two compounds in the commercial formulation and water. The system shows a very reproducible and sensitive detection, with LODs and LOQs at ppt levels for both compounds and the possibility of reducing the matrix interference in scan mode. Thanks to the high resolution and a deconvolution program, the new system is capable of resolving ions with the same nominal mass and identify unknown formulation components.

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**INFLUENCE OF SAMPLING ADSORBENTS FOR TRACKING VOLATILE METABOLITES FROM IN VITRO AND EX VIVO BIOLOGICAL SAMPLES**

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In this study, we evaluated and compared the VOCs sampling performance of different adsorbent materials for thermal desorption (TD), both in *in vitro* and *in vivo* situations, in the context of clinically-relevant samples.

Growing are the applications on VOCs from biological fluids (breath, serum, etc.), especially as possible biomarkers of specific disease states. Because of the wide variety of adsorbent materials, the tube can be packed with, it may be challenging to select the optimal tube for biological samples. Indeed, these trapping materials can be used alone or in combination and depending on the characteristics (chemical and physical), the selectivity can be tuned, as well as the sensitivity and repeatability.

Specifically, we used 7 different adsorbents (Tenax TA, Tenax GR, Carboxen 1016, Carboxen 5TD, Carboxen B, Carboxen X and Sulphicarb), packed singularly and in combination, on Fetal Bovine Serum (FBS) and human breath spiked with probe analytes. For the evaluation, we selected a mix of 19 standards (probe analytes) to monitor and compare the sensitivity and repeatability between the different adsorbents.

In the *in vitro* sampling setup, spiked FBS was used to mimic the biological matrix, and a dynamic headspace extraction was performed. For the *ex vivo*, breath was collected in Tedlar bags in which standards were successively flash-vaporized. In both cases, after extraction, the tubes were thermally desorbed on a comprehensive two-dimensional gas chromatography system coupled to a time-of-flight mass spectrometer (GC×GC-ToF MS).

For both sample matrices and in the targeted analysis on the probe VOCs, the tubes packed with Tenax TA alone resulted the most sensitive with the highest repeatability, in the range of 2-22 RSD % for breath and 2-32 RSD % for serum analysis.

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**ADVANCED AMBIENT MASS SPECTROMETRY METHODS:  
PRINCIPLES AND APPLICATIONS ON FOOD MATRICES**

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In the last decades, involved advances in the field of ionization methods for Mass Spectrometry led to the introduction of innovative “Ambient MS” techniques, so defined for the capability to generate ions directly from samples in their native form under ambient conditions. Consequently, AMS methods allowed to simplify analytical workflows by minimizing and in some cases removing sample preparation steps, and reduce analysis time since no chromatographic separation is required. The present research focused on the application of two distinct AMS approaches, namely Rapid Evaporative Ionization Mass Spectrometry (REIMS)<sup>1</sup> and Direct Analysis in Real Time (DART), for the analysis of food matrices. Those techniques led to obtain almost instantaneously unique fingerprinting of the samples of interest revealing the presence of discriminant features and specific markers useful e.g. for authenticity assessment or even for evaluation of geographical origin. For this purposes, database of MS profiles of authentic samples needed to be created and employed for the building of classification models based on statistical analysis. A comparison between the spectral profiles obtained by both AMS strategies demonstrated their capability to provide holistic profiles of the food samples. Moreover, the use of chemometric revealed their ability to be applied for the reliable identification of different samples.

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**PROCESS INTENSIFICATION IN THE BIOPHARMA INDUSTRY:  
IMPROVING PURIFICATION OF THERAPEUTIC PEPTIDES  
THROUGH MULTICOLUMN COUNTERCURRENT  
PREPARATIVE LIQUID CHROMATOGRAPHY**

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Therapeutic peptides are considered one of the most promising class of biopharmaceuticals. Their industrial production (upstream processing) has exceptionally advanced in the last ten years, especially for what regards solid-phase synthesis. However, these advancements have not been matched by equivalent improvements in purification procedures (downstream processing) which still represents the bottleneck, in terms of both cost, time and sustainability in the entire production process.

Purification of therapeutic peptides in biopharma industries is usually carried out through single-column preparative liquid chromatography and more chromatographic steps are often required. Indeed, solid-phase synthesis do not lead only to the target peptide but also to a series of product-related impurities which have very similar chemo-physical properties, as well as chromatographic behavior, to that of the target. However, single-column processes suffer of an intrinsic limitation in terms of yield-purity trade-off. This drawback can be practically alleviated through multicolumn countercurrent continuous (or semi-continuous) chromatographic approaches. The employment of two or more identical columns, working either in parallel or interconnected, allows for the internal recycle of the product into the system. The greatest advantage of this approach is that the purification process can be completely automated, with minimum need of human intervention and considerable saving of time. Meantime, the reproducibility of the process is improved, and solvent consumption is reduced [1].

This communication will show, through a series of case studies, the great potential of multicolumn countercurrent platforms to boost the downstream processing of therapeutic peptides. Particular emphasis will be given to Multicolumn Countercurrent Solvent Gradient Purification (MCSGP) and N-Rich techniques.

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**TRACKING HEME-PROTEIN INTERACTIONS IN HEALTHY AND PATHOLOGICAL HUMAN SERUM IN NATIVE CONDITIONS BY HOLLOW-FIBER FLOW FIELD-FLOW FRACTIONATION WITH MULTIDETECTION**

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Heme is an iron-containing porphyrin of vital importance since it is involved in many key processes in the human organism. Hemoproteins, such as hemoglobin and myoglobin, use it for oxygen transport, electron transport, energy generation, and chemical transformation [1]. Heme is also involved in other important enzyme systems, in the control of gene expression and post-translational modifications [2]. In addition, heme regulates differentiation and proliferation of various cell types [3].

The mechanisms underlying the interaction of heme with blood serum proteins are, however, not yet fully understood. New analytical methods able to investigate protein-heme binding in biologically representative conditions are thus required. We have previously demonstrated the ability of hollow-fiber flow field-flow fractionation (HF5), the miniaturized version of flow FFF, to size-fractionate and characterize serum proteins in native conditions [4-5]. In this work, we present HF5 with multiple spectrometric and light-scattering detection [6] for size separation of high-abundance serum proteins and selective detection of heme-bound subpopulations. Heme is found to mainly interact with serum albumin, while a low amount also binds to other proteins such as IgM. The ability to bind heme in physiological conditions is also investigated for individual serum proteins. IgG is found unable to bind heme at clinically relevant concentrations. The proposed method allows separation, quantitation, and mass/size characterization of serum high-abundance proteins, providing information of heme-protein complex stability and preferred heme-clearing pathways. The same approach could be in perspective extended to the investigation of specific heme-antibody binding, and to further studies involving other molecules of pharmaceutical/clinical interest.

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**CHARACTERIZATION OF ANACARDIC ACIDS IN SAMPLE EXTRACTS OF PISTACHIO (*Pistacia vera* L.) SHELLS BY LIQUID CHROMATOGRAPHY AND TANDEM MASS SPECTROMETRY**

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Anacardic acids (AAs, *6-n-alkyl salicylic acids*) are phenolic lipids found in the plants of the *Anacardiaceae* family. Recent studies have demonstrated that AAs are bioactive compounds not only exhibiting significant antibacterial properties [1,2] being also powerful inhibitors of tumor cells with antiproliferative and cytotoxic effects for pancreatic [3] and breast [4] cancers among the others. It seems that the health benefits may depend on the number and position of the unsaturation on the lateral alkyl chain [5]. The present work is focused on the characterization of AAs extracted from pistachio (*Pistacia vera* L.) shells by reversed-phase liquid chromatography coupled with tandem mass spectrometry using an ESI source (RPLC-ESI-MS/MS) [6]. Several alkyl chains of AAs were successfully characterized in the sample extracts of ball-milling pistachio shells. For this purpose, tandem MS analyses were carried out on standard derivatized AAs as deprotonated molecules, obtaining diagnostic product ions of the double bond position. AAs can be efficiently recovered by a green process from agri-food waste products, including dried pistachio shells. Among the others, two isomers of AA 15:1, *i.e.* 2-hydroxy-6-(pentadec-8-en-1-yl) benzoic and 2-hydroxy-6-(pentadec-6-en-1-yl) benzoic acid besides saturated and polyunsaturated phenolic lipids were identified in pistachio shells.

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**MATRIX SOLID PHASE DISPERSION MICRO EXTRACTION USING DEEP EUTECTIC SOLVENT (DES-MSPD) FOR DETERMINATION OF PESTICIDES IN TOMATOES**

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Matrix solid-phase dispersion (MSPD) involves the use of a solid, dispersing material, which is homogenized together with the sample in a mortar and loaded into a cartridge for the analytes elution with a suitable solvent. Due to its rapidity, low solvent and cost consumption, MSPD is considered a valid alternative to the Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) method, indicated as the official method for the extraction of pesticides from vegetables [1].

Deep eutectic solvents (DESs) resulted to improve pesticides extraction if added to the dispersing material and sample during the MSPD homogenization phase [2].

The aim of this work was to develop and optimize a DES-MSPD micro extraction for fourteen pesticides from tomatoes followed by HPLC-MS/MS analysis. The quantity of DES, composed by choline chloride and sesamol, to add during the MSPD homogenization phase, was optimized by Box-Behnken design together with sample-to-dispersant ratio and extraction solvent volume. Alumina and ethanol were selected as dispersing material and extraction solvent respectively, through single-factor experiments. A value of 1:4 sample-to-dispersant ratio, 900  $\mu$ L of EtOH as extraction solvent and 50  $\mu$ L of DES guaranteed the best results in terms of mean of extracted pesticides peak areas. The optimized analytical method was validated and applied to real samples considering whole tomato, tomato pulp and tomato peel.

Seven and eight of the fourteen analyzed pesticides were detected but not quantified due to their concentration lower than their limit of quantification, in whole tomato and tomato pulp respectively. In tomato peel five pesticides were quantified and their concentrations were below maximum residue levels.

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**ADVANCING MOSH/MOAH ANALYSIS TOWARDS SPECIATION  
AND CONTAMINANTS IDENTIFICATION**

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Mineral oil (MO) contamination in various food products, revealed by the presence of saturated (MOSH) and aromatic (MOAH) hydrocarbons, is a pressing topic for its potential impact on consumers' health. In particular, the presence of MOAH raises concerns for the increased risks in terms of toxicity, especially when species with 3 or more aromatic rings and low alkylation degree are suspected to be present.

Current platforms, based on the LC-GC-FID hyphenation, allow quantification of the aliphatic and aromatic contaminant fractions. This task is challenging due to matrix complexity and the presence of interferences. Moreover, it is not possible to obtain detailed qualitative information about the type of contamination present.

This contribution aims to highlight the merits of two-dimensional comprehensive gas chromatography (GC×GC) with parallel detection as additional tool, complementary to the reference methodology, for more insightful characterization of samples found positive to contamination. Instrumental configuration was designed to maximize the output of valuable qualitative and quantitative data while ensuring sustainability by minimizing the amount of sample, solvents and resources required.

**EVALUATION OF VITAMINE D LEVEL IN BIOLOGICAL FLUIDS BY USING GAS CHROMATOGRAPHY TECHNIQUE COUPLED TO TRIPLE QUADRUPOLE MASS SPECTROMETRY**

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Nowadays, vitamin D is considered a steroid hormone rather than a true vitamin, since it is mainly derived from the endogenous modification of 7-dehydrocholesterol, its precursor presents in the skin [1]. There are two main forms of vitamin D: cholecalciferol (vitamin D<sub>3</sub>) and ergocalciferol (vitamin D<sub>2</sub>). Both forms are biologically inactive. Their bioactivation occurs by the sequential action of specific hydroxylase enzymes in human organism and formation of the final product 1 $\alpha$ ,25(OH)<sub>2</sub> D. However, the major circulating metabolites of the vitamin D<sub>3</sub> and vitamin D<sub>2</sub> are 25-(OH) vitamin D<sub>3</sub> and 25-(OH) vitamin D<sub>2</sub>. Consequently, the nutritional state of the vitamin D in human organism is conventionally determined by monitoring the total content of these two hydroxylated forms, molecules with half-life of 2-3 weeks. Usually, the concentration of the active form [1 $\alpha$ ,25(OH)<sub>2</sub> vitamin D] is not determined due its very short half-life (hours).

Vitamin D status is usually measured in clinical situations by using high-performance liquid chromatography (HPLC), but the need to improve both precision and specificity of vitamin D metabolites assay has been reported in literature [2]. In order to achieve this, the proposed research study aimed to develop and optimize a gas chromatography (GC) method coupled to triple quadrupole mass spectrometry (QqQ). Particular emphasis has been paid in the optimization of the GC method in order to obtain a fast separation of target compounds. Also, a soft ionization and multiple reaction monitoring assay were developed in order to ensure high specificity, selectivity and sensibility of the analytical technique. Finally, particular attention was also dedicated to sample treatment according to the automation and miniaturization of the methodology.

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**FFF-BASED HIGH-THROUGHPUT SEQUENCE SHORTLISTING TO SUPPORT THE DEVELOPMENT OF APTAMER-BASED ANALYTICAL STRATEGIES**

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Aptamers are biomimetic receptors that are increasingly exploited for the development of optical and electrochemical aptasensors. They are selected in vitro by the SELEX (Systematic Evolution of Ligands by Exponential Enrichment) procedure. Although they are promising recognition elements, for their reliable applicability for analytical purposes, one cannot ignore sample components that cause matrix effects. This particularly applies when different SELEX-selected aptamers and related truncated sequences are available for a certain target, and the choice of the aptamer should be driven by the specific downstream application. Among the already exploited aptamer-protein systems, there is an urgent need to develop a reliable screening strategy that could help to easily and quickly select, among sequences reported in literature, the most suited for a particular analytical application. The strategy should consider the constituents in the sample matrix, the possible interferences, and the necessity of sequence functionalization with tags for subsequent transduction. In this context, our work aimed at investigating the potentialities of asymmetrical flow field-flow fractionation (AF4) with UV detection for the development of a screening method of a large number of anti-lysozyme aptamers towards lysozyme, including randomized sequences and an interfering agent (serum albumin) [1]. The possibility to work in native conditions and selectively monitor the evolution of untagged aptamer signal as a result of aptamer-protein binding makes the devised method effective as a strategy for shortlisting the most promising aptamers both in terms of affinity and in terms of selectivity, to support subsequent development of aptamer-based analytical devices.

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**A DATA DEPENDENT ACQUISITION-BASED APPROACH FOR THE IDENTIFICATION OF UNKNOWN FAST-ACTING TOXINS AND THEIR METABOLITES IN SHELLFISH**

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Cyclic imines (CI) are marine biotoxins listed among the emerging toxins of European concern characterized by fast-acting toxicity inducing a characteristic rapid death in mouse intraperitoneally injected [1]. From a chemical standpoint, CIs are macrocyclic molecules with imine and spiro-linked ether moieties, and they include spirolides, gymnodimines, and, pinnatoxins among others. Their detection in raw and processed shellfish has significantly increased in the last decades in the Mediterranean area, so raising serious concern for seafood safety. In the absence of robust toxicological data, the European Food Safety Authority has required more scientific efforts aimed to assessing their actual distribution in seafood based on validated analytical methods before establishing a maximum permitted level in seafood. As a result, currently, no regulation exists for this group of toxins [2].

In this study, a new untargeted LC-HRMS method based on data dependent acquisition has been conceived and successfully applied in combination with targeted studies for an in-depth investigation of the metabolic profile of shellfish. This approach allowed to discover several novel gymnodimine fatty acid ester metabolites, as well as to implement a new LC-HRMS-based strategy, named “backward analysis”, for discovery and identification of new toxin analogues. The implemented analytical workflow has broadened the structural diversity of gymnodimine family through the inclusion of five new analogues, gymnodimine –F, to -J. Their tentative structural characterization has been also carried out.

In parallel, the study of toxin accumulation in Mediterranean and Galician trophic chain revealed the presence of gymnodimine A in Tunisian shellfish, pinnatoxin G in mussels from the Atlantic coast of Spain (Rías Baixas) and, for the first time, pinnatoxin G in mussels from Sardinia (Tyrrhenian Sea, Italy).

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**THREE- AND FOUR-DIMENSIONAL GAS CHROMATOGRAPHY-  
MASS SPECTROMETRY BASED METHODS: WHEN AND WHY TO  
EXPLOIT**

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During the last two decades, there has been a great deal of technological evolution, on the one side, in the field of comprehensive two-dimensional gas chromatography (GC×GC), and on the other, in that of mass spectrometry (MS). Furthermore, mass analyzers can be used in sequence to form two-dimensional MS instruments. Hence, the combination of GC×GC and a single mass analyzer (single quadrupole - QMS, time-of-flight - ToFMS) will lead to a three-dimensional (3D) system, whereas the hyphenation of a GC×GC instrument with two mass analyzers in sequence (triple quadrupole - QqQMS) will lead to a four-dimensional (4D) one.

The stacking of analytical dimensions leads to highly powerful instrumentation. In themselves, GC×GC-QMS and GC×GC-ToFMS are technologies characterized by a high separation power, selectivity and sensitivity. The addition of an extra MS dimension - GC×GC-QqQMS - will boost, in particular, selectivity and sensitivity. The present research, based on the use of 3D and 4D methods, aims to provide information on when and why to exploit such approaches, with the objective of avoiding mismatches between the analytical challenges and the tool used to resolve them.

**INFRARED SPECTROSCOPY INVESTIGATION OF THE SECONDARY STRUCTURE OF GELATIN CONTAINED IN DIFFERENT GELATIN/WASTE KERATIN-BASED BIOMATERIALS**

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The possibility of using the keratin extracted from poultry feathers to produce new biomaterials is a turning point to tackle the environmental pollution caused by the disposal of these biowastes [1]. In this study, keratin was extracted from poultry feathers by microwave-assisted extraction using acetic acid. The extracted keratin was mixed with gelatin and (3-(Glycidyloxypropyl)trimethoxysilane) (GPTMS) and the blend was used to produce electrospun mats by electrospinning process (ES). Gelatin film samples containing different amounts of GPTMS were also prepared by solvent casting. A detailed infrared spectroscopy study on the secondary structure of gelatin, main protein component contained in the different gelatin/keratin-based biomaterials, was performed. We have examined how the gelatin secondary structure is affected by: (i) the process used to obtain the biomaterials (solvent casting vs. electrospinning), (ii) the concentration of GPTMS, and (iii) the keratin added. Moreover, the influence of the different conformations on the mechanical performances of the biomaterials was investigated. The results shown higher structural order in the electrospun samples with respect to gelatin films. GPTMS decreases the structural order only in gelatin films and keratin produces a decrease in  $\alpha$ -helix content in electrospun samples. Concerning the correlation between mechanical properties of the electrospun biomaterials and gelatin secondary structure, the results indicated that failure strain is directly correlated with  $\alpha$ -helix while it is inversely correlated with  $\beta$ -structures. The elastic modulus shown exact opposite correlations. Overall, ATR-IR spectroscopy has proved to be an excellent technique for the conformational study of proteins on solid samples in a simple, fast, and economic manner.

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**AN X-RAY PHOTOELECTRON SPECTROSCOPY (XPS) STUDY OF  
FRAGMENTATION PROCESS OF PLASTIC MATERIALS**

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“Plastics” are synthetic organic materials, characterized by a high degree of chemical resistance, considerable plasticity as well as versatility of performance and ease of processing. All these properties have led to the immoderate use of plastic materials with their subsequent release into the natural environment and accumulation especially in marine ecosystems<sup>1,2</sup>. Despite their resilience and resistance, plastics can undergo fragmentation processes by physical-chemical and biological agents, producing microplastics (MPs) or even nanoplastics (NPs), with consequences for organisms that populate environmental compartments. However, determining the fate of plastics materials in the environment is inherently difficult, both because the timescale of degradation and the dynamics of these processes are not clear and grey areas remain. It is well known that the fragmentation process originates from oxidation and hydrolysis of plastic materials, by solar radiation (especially the UV portion), water hydrodynamics and the activity of aquatic organisms<sup>1</sup>.

In this work, we propose the use of the X-ray Photoelectron Spectroscopy (XPS) technique for chemical characterization of different plastic materials: polyethylene terephthalate (PET), polystyrene (PS) and polyethylene (PE), before and after a treatment aiming at simulating environmental conditions determining oxidation/hydrolysis processes, to understand how plastics change towards fragmentation. In brief, plastic standard samples (plastic pellets with a size of around 1 cm<sup>2</sup>) are artificially weathered for different time intervals by the exposure to controlled UV radiation in water solution at hydrodynamic conditions (under stirring, 400 rpm). After the treatment, the plastic standard samples are analyzed by XPS. Preliminary results show that proceeding the treatment, there is an increase of oxygen-containing functional groups (namely, C-O, C=O and O-C=O) on the plastics surface suggesting a partial degradation of the polymeric materials, under these conditions. XPS proves to be a useful technique for the characterization of plastic materials, to obtain meaningful information about their fragmentation.

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**ANALYTICAL SPECTROSCOPY INVESTIGATIONS IN THE GALVANIC INDUSTRY TO STUDY INTERMETALLIC DIFFUSION**

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Analytical spectroscopy techniques (such as XRF, EDX, EIS) are used in the galvanic field for a wide variety of purposes, especially to assess the quality and the stability of the metal deposits. In this case, our work was focused on developing and validating an easy-to-use and cheap method to investigate the diffusion coefficient, a critical chemical-physical parameter for studying and foreseeing the behaviour of multi-layers systems, in terms of corrosion and stress resistance, conductivity and colour. As it stands, the go-to methods are the Auger Electron Spectroscopy (AES), FIB-SEM/TEM systems and XPS. All these techniques use expensive equipment, resulting unaffordable for small or medium-sized enterprises and provide data difficult to interpret. The proposed method is based on Energy Dispersive X-ray Spectroscopy (EDS/EDX) measurements coupled with Monte Carlo simulations to obtain the thickness of diffused metal via the K-ratio method [1]. The set-up used is easier to learn and it is at least ten times cheaper compared to those listed above. To validate the technique, coefficient diffusions of Copper in Gold at four different temperatures (100 °C, 133 °C, 167 °C, 200 °C) were measured. The results obtained on electrodeposited samples, consisted of 400 nm approx. of Gold on top of a Copper substrate, agree with those found in literature [2]. Then, systems having a barrier layer of 400 nm approx (Ni, NiP, PdFe, White Bronze and PdNi) between Gold and Copper were investigated, measuring the diffusion coefficient of copper and the barrier layer metals in Gold. At the same time XRD, XRF and colorimetric analysis were carried out to better understand the intermetallic diffusion phenomenon. The authors acknowledge Regione Toscana POR CreO FESR 2014-2020 – azione 1.1.5 sub-azione a1 – Bando 1 “Progetti Strategici di ricerca e sviluppo” which made possible the projects “A.C.A.L. 4.0”, “GoodGalv”, “GIGA 4.0” and “A.M.P.E.R.E.” and MUR and EU-FSE for financial support of the PhD fellowship PON Research and Innovation 2014-2020 (D.M 1061/2021) XXXVII Cycle in Chemical Sciences.

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**EXPLORING PLASMONIC-BASED IMAGING SENSOR FOR ULTRASENSITIVE MOLECULAR DIAGNOSTICS: THE ROAD TO LIQUID BIOPSY**

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The detection of diagnostic biomarkers (i.e. circulating tumor DNAs, cell-free DNA, microRNAs and proteins) present in extremely low concentrations in body fluids, promises to enhance the accuracy in early cancer diagnosis as liquid biopsy approach [1]. To extend the applicability of the Surface Plasmon Resonance Imaging (SPRI) sensor in detecting biomarkers at ultralow levels in a clinical setting, we combine the advantages offered by Peptide Nucleic Acid (PNA) probes in improving the selectivity when targeting complementary DNA or RNA sequences, with the enhancement of the SPR signal obtained by using functionalized gold nanoparticles, minimizing at the same time non-specific adsorptions from biofluids by adopting appropriate antifouling surface. By implementing a sandwich assay, it was possible to reveal ~1 attomolar KRAS mutated DNA circulating in 40  $\mu$ L of plasma from cancer patients, greatly simplifying liquid biopsy workflow [2]. More recently, a similar approach is envisaged to be applied for non-invasive prenatal fetal sex determination based on the detection of a Y-chromosome specific sequence (single-gene SRY) in cell-free fetal DNA from maternal plasma, even at the earliest gestational age [3]. Possibilities offered in the detection of proteins as cancer biomarkers will be also discussed. In this direction, a new anti-fouling PEG-pentamer carboxybetaine-based surface has demonstrated its applicability in plasma for the SPRI biosensing of human Arginase [4]. The proposed SPRI platform shows broad potential to detect biomarkers with inexpensive, non-invasive, highly sensitive, and highly specific advantages.

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**POLYMER COMPOSITION ANALYSIS OF PLASTIC DEBRIS  
INGESTED BY LOGGERHEAD TURTLES (*CARETTA CARETTA*) IN  
SOUTHERN TYRRHENIAN SEA THROUGH ATR-FTIR  
SPECTROSCOPY**

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The ingestion of anthropogenic plastic debris by marine wildlife is widespread in the Mediterranean Sea. The endangered status (in the IUCN Red List) of Loggerhead turtle (*Caretta caretta*, Linnaeus, 1758) is a consequence of its vulnerability. In this study, macro-/meso-plastics (5-170 mm) collected from faeces of twelve loggerhead turtles rescued (live) in the Aeolian Archipelago (Southern Tyrrhenian Sea, Italy) were analyzed by size, weight, shape, color and polymer type through Attenuated Total Reflectance Fourier-Transform Infrared Spectroscopy (ATR-FTIR). The defecation rate during hospitalization (7-14 days) varied among turtles (from 0.08-0.58). The mean number of plastic expulsions ( $2.7 \pm 1.8$  items for turtle) was higher during the 5<sup>th</sup> day of hospitalization (Kruskal-Wallis test,  $P = 0.01$ ). However, the mean number of plastic-like items defecated during the common days of hospitalization did not vary among turtles (Kruskal-Wallis test,  $P > 0.05$ ). All turtles were found to have ingested plastic. A total of 114 debris items were recovered from their faeces, 113 of which were identified as plastic. Their color was mostly white-transparent (64.9%) and light (19.3%). Shape was mainly fragments (52.6%), sheets (38.6%), followed by nylon, net-fragments, elastic plastic, foamed plastic and industrial granules (8.8%). Meso-plastics (5-25 mm) represented 72% of the total number of debris and were found more frequently in turtle with Curved Carapace Length (CCL)  $\leq 60$  cm (CCL = 30-60 cm,  $n = 5$ ) than those with CCL  $> 60$  cm (CCL = 60-71 cm,  $n = 7$ ). Plastic items were composed mainly of polyethylene (48.2%) and polypropylene (34.2%). Polypropylene ( $R^2 = 0.95$ ,  $P < 0.001$ ) and

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polyisoprene ( $R^2 = 0.45$ ,  $P = 0.017$ ) were more common in meso-plastics while polyethylene ( $R^2 = 0.44$ ,  $P < 0.01$ ) in macro-plastics. Finally, high-density polyethylene, polyvinyl chloride, polyamide and polyurethane were also found in some turtles. This study reveals high spreads of plastic contamination in faeces of both turtles with  $CCL \leq 60$  cm and  $CCL > 60$  cm, particularly vulnerable to the increasing quantity of floating plastic into their foraging sites highlighting the need of further research to associate debris ingestion with turtle diet and their size.

C. Bruno, M. F. Blasi, D. Mattei, L. Martellone, E. Brancaleone, S. Savoca, Gabriele Favero, under review in Marine Environmental Research.



**EXPLOITING p-X-RAY FLUORESCENCE AS METHOD FOR THE ANALYSIS OF ANCIENT POTTERY**

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Portable X-ray fluorescence (p-XRF) spectrometers are valuable instruments for identifying and quantifying elements having  $Z$  higher than 11 in a quick and easy way, with almost no sample preparation and, in the case of standardless instrument, with no requirements for calibration procedures. Another advantage is the ease of use of these spectrometers that makes them suitable also for non-expert users.

For these reasons, p-XRF are applied in various fields ranging from material science to cultural heritage.

However, the evaluation of the analytical performances of such instruments, should be accurately investigated in order to ascertaining the reliability of the quantification of the elements constituting the investigated samples.

In this work limit of detection, limit of quantification, precision, accuracy and linearity were determined for method validation. The analyses were performed on clays and pottery by a handheld standardless XRF spectrometer.

In order to evaluate the analytical performance of the spectrometer, four certified reference clay samples were analyzed as powder, adopting different preset calibration curves suitable for environmental and mineral samples.

The same calibrations were also adopted for determining the composition of pure  $\text{SiO}_2$ ,  $\text{Al}_2\text{O}_3$ ,  $\text{CaO}$ ,  $\text{MgO}$ , and their mixtures with different compositions, and for quantifying the composition of Sardinian clays previously characterized by inductively coupled plasma optical emission spectroscopy (ICP-OES).

Adopting proper pre-set calibrations allows obtaining good results in terms of precision (relative standard deviations  $< 0.5\%$ ) and accuracy (% error  $< 1\%$ ) in the case of pure oxides. Very good agreement between p-XRF and ICP-OES results on clays was obtained for Zn, As, Rb, Sr, K and Fe with RSD  $< 5\%$ . Good linearity was achieved for silica-based mixtures ( $R^2 > 0.99$ ). Finally, the method was applied for highlighting differences in terms of composition on pottery fragments and powders.

A selection of the results obtained on ancient pottery fragments from the Punic-Roman period, found in the archaeological area of Nora (CA) will be presented together with a preliminary attempt of provenance analysis.

**COATINGS THICKNESS DETERMINATION USING SEM AND ENERGY DISPERSIVE X-RAY SPECTROSCOPIES WITH A STANDARDLESS APPROACH**

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Among the numerous techniques for the thickness determination none allows fast, inexpensive, versatile and non-destructive analysis that is required in the industrial quality control process of many applications, especially for metal coatings. Even if X-Ray Fluorescence spectroscopy (XRF) is a widespread technique in this field, the thickness determination of multilayer coatings is not straightforward because of the complex physics involved and the high matrix effects. Errors exceeding 10% are typical but not always acceptable. XRF quantification is based on the Fundamental Parameters (FP) approach, sometimes adjusted with empirical measurements. Due to the difficulties in the standards preparation, certified samples are used but with two major drawbacks: (i) there is not the availability of standards for any possible coating composition and architecture and (ii) commercial standard are quite expensive. In this study, we used a semiquantitative approach for the coating thickness measurement based on the construction of calibration curves through simulated EDS and XRF spectra obtained with Monte Carlo algorithm <sup>[1]</sup>. Multivariate data analysis was performed to consider the effect of the multilayer structure simultaneously <sup>[2]</sup>. We assessed the accuracy of the method by comparing the results with XRF-FP and FIB-SEM analysis. Moreover, the pros and cons of the use a bulk material as reference (peak ratio, PR, method) or different emission lines of the sample (internal standard) were evaluated.

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**ANALYTICAL CHARACTERIZATION AND ANTIMICROBIAL ACTIVITY OF GREEN ZEOLITE-THYMOL COMPOSITES**

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Bacterial resistance to multiple antibiotics is a huge health problem [1]. In this respect, many plant extracts have been tested worldwide as potential sources of novel antimicrobial compounds [2].

In this work, the development, analytical characterization and bioactivity of zeolite-thymol composites, obtained using wet, semi-dry and dry processes, were carried out in order to obtain sustainable and powerful antimicrobial additives [3]. Analytical characterization by means of FT-IR, XRD, DSC, TGA and SEM was carried out to gain comprehensive information on the chemical-physical, thermal, and morphological features of the composites. GC-MS analyses allowed quantifying the active molecule loaded in the zeolite, its release and stability over time. Among the three loading procedures, the dry (i.e., solvent-free) approach allowed to reach the highest thymol content and loading efficiency ( $49.8\pm 1.6\%$  and  $99.6\pm 1.2\%$ , respectively). Therefore, the bioactive surface of composites obtained by this method was assayed for its antimicrobial activity against four microbial strains belonging to *S. aureus*, *E. coli*, *P. aeruginosa* and *C. albicans* species. The higher antimicrobial activity produced by the solvent-free composite in comparison with that of pure thymol, at the same thymol concentration, was ascribed to the large interfacial contact between the composite and the bacterial target. This feature, together with its enhanced storage stability, suggested that this composite could be employed as effective additives for the development of antimicrobial biointerfaces for food, home and personal care applications.

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**THE EFFECT OF THE ARTIFICIAL SALIVA COMPOSITION FOR CORROSION STUDIES: EVIDENCE FROM XPS ANALYSES.**

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Human saliva is unstable outside the oral cavity and its composition is strongly variable from person to person and it changes during the day [1]. Therefore, several formulations have been proposed for electrochemical and corrosion investigations. In our recent studies [2] the corrosion performance of CuZn37 in contact with three different saliva formulations was investigated; it was found that the corrosion rate  $v_{\text{corr}}$  varied in the order  $D > C-B > S$ . As the three solutions Darvell (D), [3] Carter – Brugirard (C-B) [4] and SALMO [5] contain similar amounts of chlorides, thiocyanates, phosphates and carbonates, the difference in  $v_{\text{corr}}$  might be attributed to the content of organic compounds: Darvell solution contains urea, lactic acid, trisodium citrate, and uric acid; SALMO solution contains urea and glycine; Carter-Brugirard solution contains only urea. The present work focuses on the surface characterization of CuZn37 brass exposed to different saliva solutions by X-ray photoelectron spectroscopy (XPS), aiming to correlate the surface composition of the film to the corrosion behaviour of CuZn37. After exposure to SALMO and C-B solutions, the high-resolution spectra of S 2p and P 2p show a single doublet at BE 163.4 (0.1) eV for sulphur assigned to  $\text{SCN}^-$ ; the P 2p signal is revealed at 134.3 (0.1) eV, attributed to the formation of zinc-phosphate. In the case of CuZn37 in contact with D solution, the P signal is absent and the Zn intensity is much lower than in the other two solutions. In the S2p spectrum a second component at 161.9 (0.1) eV is revealed in addition to the one assigned to CuSCN component. The Zn / Cu ratio in the surface film, was found much lower for the Darvell solution. These results will be discussed in the light of the role of organic compounds that might act as ligands and affect the film composition.

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# Poster

**TRIACYCLGLYCEROLS CHARACTERIZATION OF TUNA FISH OILS FROM GREEN PRODUCTION BY HPLC/HRMS AND GC/MS.**

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Food waste generated in great quantities worldwide is a current point in the view of a more sustainable circular economy. Depending on the type of food a different degree of wastes is generated, taking into consideration both food chain and industrial processing. The residues produced by the various supply chains in several cases are still characterized by a good content of bioactive molecules which, properly recovered, have the potential to be reintroduced at different stages of the production cycles of other products, often reinforcing their intrinsic value. For this reason, are always welcome the development of new protocols to recover molecules of nutritional/nutraceutical interest from by-products and waste of the agri-food and industrial production chain. The simplest and less invasive recovery procedures are of course preferable as they should do not introduce bioactive principles modification, and reduce the presence of xenobiotic substances. The chemical characterization of the extracted oil has been performed determining fatty acids methyl esters analysed by GC/MS, and triglycerides (TAGs) determined by an HPLC/HRMS approach based on a ballistic gradient. Both the results obtained have been compared with literature data [2] based on commercial tuna fish oil, and are in good agreement. On the other hand, several other TAGs have been identified in our study, and, in a semi quantitative approach, their relative abundances have been used to determine fatty acid composition by means of an in-silico saponification approach [3]. The described approach allowed to individuate and determine 84 different TAGs. The in-silico saponification, based on the identified TAGs and their relative abundance, gave relative percentual of fatty acids that are in fair agreement with the fatty acid percentages determined by the traditional FAMEs protocol, suggesting a good and correct identification/quantification of the TAGs.

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**ANALYTICAL ASSESSMENT OF MEAT SHELF LIFE:  
EVALUATION OF THE EFFECT OF NOVEL BIOBASED  
ANTIOXIDANT TREATMENTS**

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Oxidative stress is one of the main factors affecting food stability and its sensory properties. Additives and preservatives are generally used by producers as food supplement for shelf-life improvement, although they are reported to show adverse impact on human health. This work aims at evaluating the effect of novel biobased antioxidant treatments, deriving from natural matrices, on meat shelf-life. The proposed solutions were prepared in form of edible film and spray based on natural polysaccharides. Experiments were performed on different types of meat, such as hamburger, beef steak, horse fillet. Analytical evaluation was carried out by colorimetry (CIELAB space) providing useful information about the color variation ( $\Delta E$ ) during time, and by Oxitest reactor, an automated analytical tool performing accelerated shelf-life analysis measuring fat oxidation process. The combination of these analytical techniques allowed to monitor the effects of antioxidant-enriched coatings on meat shelf-life providing promising results. Treated meats showed highest resistance to oxidation stress and lower color variation compared to untreated samples. Moreover, some color coordinates were found out in altered samples and selected as possible markers useful to follow the oxidation phenomenon during time. In addition, bioactive molecules extracted from agro-industrial by-products were considered, according to the new circular economy principles.

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**DEVELOPMENT OF METHODS FOR CHECKING THE FRESHNESS OF FISH BASED ON GCxGC-MS AND LED MULTISPECTRAL IMAGING**

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During conservation, fish may be exposed to various chemical processes caused by bacterial activity, such as conversion of amino acids to biogenic amines, breakdown of lipids into individual fatty acids (hydrolytic rancidity), or degradation to alcohols, aldehydes, and ketones (oxidative rancidity). Traditional analytical methods are effective for their determination, but they usually exploit invasive sampling procedures and multiple-step pre-treatments.

We carried out a feasibility study to distinguish fresh from spoiled sea bream (*Sparus aurata*) samples under two storage conditions: no storage and refrigeration for 7 days. The *first method* was based on the search for volatile compounds and fatty acids through GCxGC-TOF analysis. Analytes from fish gills, scales, and eyes were extracted using noninvasive EVA strips functionalized with ion exchange resins. The identified compounds were then analyzed using multivariate methods (PCA and PLS-DA), allowing for the detection of differences between the two storage times.

The *second method* used LED Multi-spectral Imaging to detect absorption differences between fresh and preserved samples when the sample's surface was irradiated by electromagnetic waves covering the spectral range from UV to NIR (8 wavelengths). The analysis is fast, cheap and leaves the product intact. The results indicated that the greatest differences were found in the eyes of the fish. Greater absorption of NIR waves throughout the organ was explained by the abundance of carboxylic acids and alcohols. Under UV radiation, there was a clouding of the iris due to protein degradation, as revealed by the presence of free amino acids.

The work is financed by Ministero della salute (Ricerca Finalizzata 2016 - GR-2016-02363722 - The use of LED multi-spectral imaging for fast and innovative indexing of seafood preservation).



**EVALUATION OF COUMARINS, FUROCOUMARINS AND POLYMETHOXYFLAVONES CONTENT IN FLAVORED BEVERAGES BY MEANS OF HPLC-MS/MS WITH THE SUPPORT OF LINEAR RETENTION INDEX APPROACH**

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The present research is focused on the development of an innovative analytical method involving the use of a HPLC-MS/MS instrumentation, applied in combination with the Linear Retention Index (LRI) approach, for the quantification of 37 compounds belonging to Coumarins (Cs), Furocoumarins (FCs) and polymethoxyflavones (PMFs). These compounds show similar chemical structures and are shortly named Oxygen Heterocyclic Compounds (OHCs).

In detail, the method was applied on flavoured citrus beverages. Flavours are widespread used to enhance beverage taste and aroma by adding essential oil, juice or peel during the preparation. The OHC profile can vary according to the species (bergamot, lemon, sweet and bitter orange, mandarin, ecc.) and the part of the fruit considered (peel or pulp).

Several beneficial effects of OHCs have been demonstrated. However, recent studies are focused on the evaluation of the adverse effects as a consequence of their dietary intake. For this reason, the European Parliament and the European Food Safety Authority (EFSA) released regulations and opinions on the content of OHCs in several foods and beverages. Currently, only coumarin is subject to restriction by the *Regulation (EC) No 1334/2008* of the European Parliament [1] and an official limit about the maximum content of FCs in food is still missing.

Thanks to the high sensitivity of the tandem mass spectrometry detection in Multiple Reaction Monitoring (MRM) mode, the Limits of Quantifications (LOQs) for the target compounds resulted very low (at the pg level). This method is useful to quantify OHCs, especially FCs, contained at trace levels in several beverages in order to evaluate the dose-effects relationship and to give informative data for new opinions and regulations in this field.

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**SOLID PHASE MICROEXTRACTION ONLINE COUPLED TO DIRECT ANALYSIS IN REAL TIME FOR THE DIFFERENTIATION OF HIGH-QUALITY ITALIAN EXTRA VIRGIN OLIVE OILS**

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Extra Virgin Olive Oil (EVOO) is a precious source of “healthy” fats in Mediterranean diet. Due to its high commercial value, related to its unique nutritional and sensory properties, is a primary target for fraudulent activities. Current trend proposes the use of fast and high-efficient methods to achieve specific fingerprinting of EVOO and build database useful for the detection of adulterations and mislabelling practices. Both targeted and untargeted approaches have been widely exploited to achieve a similar task, focusing on specific constituents or the entire matrix. They often require a dedicated sample preparation, the use of quite expensive instrumental platforms and/or time-consuming analytical procedure<sup>1,2</sup>.

Within this context, the present research focuses on the combination of ambient mass spectrometry (AMS), specifically Direct Analysis in Real Time (DART) with chemometrics for the building of statistical models aiming to detect similarity and differences between multiple groups of EVOOs. A targeted approach involving the extraction of phenolic compounds by SPME (solid phase microextraction) prior of the analysis was exploited to highlight differences between EVOOs coming from different Italian regions, based on minor components responsible of both healthy and organoleptic features. Furthermore, the obtained results were compared with an untargeted strategy based on the analysis of EVOOs in their native form, in terms of sample clusterization into the chemometric space and identification reliability of “unknown” samples.

The coupling of the DART source with a single quadrupole MS analyzer allowed for the development of a cost-effective and user-friendly approach for the rapid analysis of EVOOs.

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## INVESTIGATION OF LIPIDS COMPOSITION IN NOVEL SPECIES OF MICROALGAE BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY COUPLED TO MASS SPECTROMETRY AND LINEAR RETENTION INDEX APPROACHES

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Microalgae as microscopic phytoplanktons is a source of proteins, carbohydrates, vitamins and lipids. Owing to their employment as nutraceuticals in many food matrices, various researches are focused on the identification of new species of microalgae beside the evaluation of their chemical content. In the present study, five novel species of Mediterranean microalgae were examined through HPLC-MS technique for their composition in lipids, polyphenols, carotenoids, chlorophylls and tocopherols. Regarding polyphenols, a poor profile was obtained for all the samples despite the employment of different extraction solvents with increasing polarity. On the other hand, carotenoids and tocopherols showed an interesting profile, which represents the unique fraction responsible for the obtained antioxidant activity. An heterogenous and rich profile of lipids was perceived among the studied species, containing mono-, di-, and triglycerides and a wide range of polar lipid classes, such as sulfoquinovosyldiacylglycerol, monogalactosyldiacylglycerol and digalactosyldiacylglycerol. Due to the high complexity of the lipid fraction, a high-resolution chromatographic approach was exploited in order to maximize the separation of lipid compounds. Specifically, two serially-coupled partially porous columns were used to enhance the chromatographic separation. A previously implemented analytical method based on the application of Linear Retention Index in LC<sup>1</sup> was adapted to this discovered species. Furthermore, an enhancement of the separation of the polar compounds was attained with a slight modification of the mobile phase. The present microalgae can be potentially employed as biological integrators.

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**EXTRACTION, CHARACTERIZATION, AND EVALUATION THROUGH *IN VITRO* STUDIES OF THE ANTHELMINTIC EFFICACY OF PASTEUR PLANT SPECIES FOR BIOLOGICAL CONTROL OF SHEEP GASTROINTESTINAL NEMATODES (GIN)**

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Gastrointestinal nematode (GIN) infection jeopardizes the health and welfare of livestock and is commonly associated with economic losses, especially due to subclinical diseases [1]. In recent years, important advances have been made in the biological control of GIN in ruminants [2-3]. Although these measures are still relatively little used in practice, interest is growing due to drug resistance, increased demand for organic products and legislation regulating and restricting the use of anthelmintic drugs [4]. The purpose of this study was to extract, characterize and evaluate the *in vitro* anthelmintic activity of aqueous, alcoholic and hydroalcoholic extracts of 28 plants sampled on pastures in southern Italy. Extracts were obtained by the conventional maceration technique. The total phenolic content (TPC, Folin-Ciocalteu assay) of all extract samples was preliminarily characterised. The egg hatching test (EHT) was performed to estimate the *in vitro* anthelmintic efficacy of plant extracts using GIN eggs from sheep naturally infected with the genera *Haemonchus contortus*, *Teladorsagia circumcincta* and *Trichostrongylus colubriformis*. Finally, extracts that showed significant anthelmintic efficacy *in vitro* were characterised by LC-MS. Each extract that showed high inhibition of egg hatching was tested *in vitro*: MDBK cell lines (bovine kidney cells) were used. All extracts showed significant TPC. Each extract was tested with EHT at decreasing concentrations of 28.0, 98.0 and 40.0 mg·mL<sup>-1</sup> for aqueous, ethanolic and hydroalcoholic extracts, respectively. The results indicated that, in particular, the extracts of *Borago officinalis* L. and *Malva sylvestris* L. caused a high inhibition of egg hatching within 48 hours of exposure, showing efficacy (≥98%) at the first two highest concentrations. The first experiments on *in vitro* cell cultures were tested at different concentrations and times: 10, 100, 500 and 1000 g·mL<sup>-1</sup> for times of 24, 48 and 72h. All extracts were cytocompatible, except for a few points where we used the highest concentration: for *B. officinalis* 100, 500 g·mL<sup>-1</sup> at 72h and for *M. sylvestris* 1000 g·mL<sup>-1</sup> at 72h. Furthermore, the results obtained from the *M. sylvestris* extract suggest a proliferative effect.

Characterisation of the extracts showed the presence of compounds of the tannin class, flavonoids, glycosides, alkaloids and terpenoids. Field application with *in vivo* studies would lead to confirm the results obtained from *in vitro* tests, evaluating the therapeutic potential and future applicability with specific plant crops in pastures to achieve year-round "helminth-free" sheep flocks.

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**TWO EGGS A DAY ARE GOOD: EXOGENOUS CHOLESTEROL IS NO LONGER A HUMAN HEALTH CONCERN.**

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Chicken egg is one of the most complete natural foods from a nutritional point of view, as it provides the most important macronutrients for human nutrition: proteins, mainly in the yolk, but also in the egg white, fats mainly in the yolk, few carbohydrates, vitamins and minerals. However, it was considered a major dietary source of cholesterol. The first research on determining the cholesterol content in eggs led to a result of 274 mg/egg. These data have been used for several years as the absolute cholesterol content in eggs. The 2015-2020 Dietary Guidelines for Americans recommended the consumption of eggs as part of a healthy diet and abolished the 300 mg/day dietary cholesterol intake limit in American dietary guidelines since 1968. Already in 2001, Naviglio et al. focused their attention on free cholesterol levels in eggs. In the literature, several articles report that healthy subjects invited to eat one or more eggs a day for three or four weeks were subsequently monitored for their blood cholesterol level. At the end of the trial, the results showed that the cholesterol content in the blood had not changed. Therefore, there is no contraindication to the intake of eggs, except in particular situations to be analyzed case by case.

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## CANNABIS SATIVA PRODUCTS AS A VALUABLE SOURCE OF NUTRACEUTICALS AND FUNCTIONAL FOOD INGREDIENTS

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*Cannabis sativa* L. is nowadays considered a multipurpose, sustainable, and low environmental impact crop. The term hemp is used to refer to *Cannabis sativa* cultivars grown for industrial purposes, characterized by low levels of THC. Specifically, varieties showing a THC content <0.2% can be freely used in Europe (EU Regulation 1307/2013) [1].

Despite the well-consolidated use as a source of textile fiber, in the last decades, a growing interest in the nutritional value and nutraceutical properties of industrial hemp has been registered.

In regard to this, hemp seeds have been reported to have a highly favourable composition in polyunsaturated fatty acids (PUFA), as linoleic acid and  $\alpha$ -linolenic acid, high-quality and easily digestible proteins rich in essential amino acids, vitamins, antioxidant molecules (e.g. polyphenols) and minerals. As a consequence, hemp seeds and their derived products may represent a promising source of functional ingredients for the production of nutraceuticals.

In this research, the composition of hemp seed oils and flours has been investigated with particular emphasis on the lipid components, by gas chromatography coupled to mass spectrometry and flame ionization detection and liquid chromatography coupled to mass spectrometry (LC-MS). Furthermore, a recently introduced linear retention index (LRI) approach in LC coupled to mass spectrometry detection has been successfully employed as a useful tool for the reliable identification of triacylglycerol components. A total of 30 fatty acids and 62 glycerolipids were positively identified in the investigated samples. Relative quantitative analyses confirmed linoleic acid as the most abundant component (50-55%). A favourable  $\omega 6/\omega 3$  ratio, around 4:1, was also measured in hemp-derived products, with the  $\alpha$ -linolenic acid around 12-14%. Finally,  $\gamma$ -linolenic acid was found to be higher than 1.70%.

[1] EU Regulation 1307/2013 Establishing Rules for Direct Payments to Farmers under Support Schemes within the Framework of the Common Agricultural Policy and Repealing Council Regulation (EC) No 637/2008 and Council Regulation (EC) No 73/2009.



**AN INNOVATIVE NON-INVASIVE METHOD FOR THE AUTHENTICATION OF APPLE CULTIVARS THROUGH COMPREHENSIVE GAS CHROMATOGRAPHY**

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Food authentication is critical to safeguarding consumers, dealers, and producers against fraud. Although various approaches have been developed employing a variety of analytical techniques, the majority of them require sample destruction and do not allow for in situ sampling or analysis. We developed and tested a new non-invasive analytical methodology for targeted and untargeted metabolomics for food authentication, which allowed an accurate quantification of hundreds of molecules from several varieties of apple surface<sup>1</sup>.

Small molecules are adsorbed from the surface of the food product using a functionalized strip, followed by the detection of the desorbed analytes using bidimensional gas chromatography coupled to mass spectrometry. The method was analytically validated and then applied for the classification of five different apple varieties. Molecular concentrations obtained from the analysis of 44 apples were used to identify markers for apple cultivars or, in combination with machine learning techniques, to perform cultivar classification.

The overall reproducibility of the method was very good, showing a good coefficient of variation for both targeted and untargeted analysis. Our results indicated that the surface of the apple was sufficiently homogeneous from a chemical point of view, suggesting the potential use of the surface sampling method for the analysis of apples. The analysis performed to distinguish different apple varieties (Fuji, Gala, Golden, Envy and Ambrosia) allowed the identification of nearly 800 molecules, including esters, alcohols, sugars, terpenes, fatty acids, alkanes, and aldehydes. Some of them were associated with the sensory quality of apple fruit. The sampling technique combined with a gas chromatography–mass spectrometry method and a machine learning approach was able to correctly classify all samples correctly. In addition, the method was also used to detect pesticides and the following molecules were found in almost all samples: chlorpyrifosmethyl, deltamethrin, and malathion. The proposed approach not only showed very good analytical performance, but also proved to be suitable for non-invasive food authentication and pesticide residue analysis.

The proposed method provided excellent analytical performance, demonstrating its suitability for non-invasive food authentication and pesticide residue analysis.

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## **ANALYTICAL CHARACTERIZATION OF CONTAMINANTS MIGRATING FROM FOOD CONTACT MATERIALS**

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Since many years, high attention has been paid to migration of contaminants from food contact materials (FCM) to products. FCM include all materials and articles intended to come into contact with food. They can be made of different raw materials combined with other substances, added to enhance stability and mechanical properties, and that can be found as undesirable compounds into foodstuffs. Furthermore, FCMs can also contain other substances that originate from reaction by-products, degradation process, oligomers, impurities, and molecules from chemical reactions between the materials and the foodstuff. All these substances are called non-intentionally added substances (NIAS). Analysis of NIAS has proven to be very challenging, since their identity is often not known. Consequently, innovative analytical approaches based on untargeted analysis, for their detection and identification, and of emerging contaminants, are in continuous-development. The aim of this research has been to exploit gas chromatography coupled with mass spectrometry to investigate potential migrations from complex units composed by primary, secondary, and tertiary plastic-based packaging. Moreover, in accordance with the European Regulation 2019/904 (entered in force in January 2022) on the reduction of the impact of plastic products on the environment, research was focused on the investigation of possible migrating substances from bio-based materials, such as those used for single use items (paper coffee cups, compostable plates, etc.). The study also involved biodegradable articles produced from a composite material derived from PBAT reinforced with natural fillers.

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[3] Regulation (EU) No 10/2011 on plastic materials and articles intended to come into contact with food. <https://eur-lex.europa.eu/eli/reg/2011/10/oj>

**IDENTIFICATION OF VOLATILE QUALITY MARKERS IN MINIMALLY PROCESSED FRESH-CUT ICEBERG LETTUCE BY A VOLATOLOMIC APPROACH**

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Fresh-cut produce industry is rapidly growing in response to an increased consumer demand of ready-to-use products. However, during the fresh-cut lettuce processes, mechanical wounding could cause disruption of cells, which induces physiological responses such as an increase in respiration rate and alternative enzyme pathways. These effects are associated with production of phenols and volatile organic compounds (VOCs) [i]. In this study, a volatolomic approach is proposed for the study of fresh cut iceberg lettuce in passive and active (O<sub>2</sub> <5%) modified atmosphere packaging (MAP), with the aim of identifying target molecules that could signal a quality food decrease, together with a VOC characterization of fresh cut iceberg lettuce aroma correlated with the type of MAP used during the storage. VOC contents have been monitored by headspace solid-phase microextraction (HS-SPME) followed by gas chromatography - mass spectrometry (GC-MS) analysis [ii]. The quality of products stored at 4°C have been evaluated at 0, 3, 7, 10 and 14 days.

A semi-quantitative analysis of both batches has shown that the most numerous class of compounds is represented by hydrocarbons, in particular alkanes. In the samples with active MAP, the presence of some terpenes that were not identified in the samples with passive MAP is also highlighted. Finally, an accurate analysis of degradation products has been carried out and discussed.

<sup>1</sup> M.E. Saltveit, 21 (2000) Postharvest Biology and Technology 61–69.

<sup>1</sup> G. Spadaccino, L. Frabboni, F. Petruzzi, G. Disciglio, A. Mentana, D. Nardiello, M. Quinto, 202 (2021) Journal of Pharmaceutical and Biomedical Analysis 114167

**DEVELOPMENT AND VALIDATION OF TWO METHODS FOR MULTI-ELEMENT ANALYSIS OF CEREAL-BASED FOODS INTENDED FOR INFANTS AND YOUNG CHILDREN BY ICP-OES AND ICP-MS.**

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The promotion and improvement of appropriate feeding practices for infants and young children represents a main topic in food action, so the monitoring of nutritional requirements and limits for contaminants is necessary for ensuring both the nutritional adequacy and safety [1]. In this research study a validated approach for the simultaneous determination of 30 elements – 24 trace elements (Al, Cr, Fe, Co, Ni, V, Li, Zn, Cu, Be, As, Mn, Mo, Ag, Se, Sr, Sb, Sn, Ba, Cd, Hg, Pb, Tl, U) and 6 oligoelements (Ca, Mg, Na, K, P, S) – using inductively coupled plasma optical emission spectrometry (ICP-OES) and inductively coupled plasma mass spectrometry (ICP-MS) after microwave digestion, is presented. For sample preparation various digestion methods were evaluated and the consumption of HNO<sub>3</sub> and H<sub>2</sub>O<sub>2</sub> was minimized through an optimized mixture design. The analytical methods were validated with good results in terms of sensitivity (limits of quantification in matrix in the range 0.050-1300 ng g<sup>-1</sup>), linearity (R<sup>2</sup> > 0.999), precision (RSD: 1.1-28.0%), and trueness (recoveries from 78 to 111%). Three certified reference materials, Rice Flour SRM 1568b, Wheat flour SRM 1567b and Typical Diet SRM 1548a (NIST), were used for the validation of both methods. In addition, the procedures were applied to the analysis of 60 specific formulations designed for the dietary management of infants and young children (rice and rice pasta, tapioca, oat, corn, quinoa, millet, spelt, semolina, durum and soft wheat pasta, multigrain snacks), available on Italian market. The contents of regulated toxic elements (Pb, Cd) were found below the maximum levels in all samples, except for a rice pudding sample with a Cd concentration above 40 ng g<sup>-1</sup> (64.8±18.4 ng g<sup>-1</sup>) [2]. The total As concentration range was 2-273 ng g<sup>-1</sup>; in 13 samples with As concentration above 100 ng g<sup>-1</sup>, the determination of inorganic arsenic was also performed by HPLC-ICP-MS for an accurate As speciation. Similarly, the amounts of micronutrients and minerals met the compositional requirements proposed by the CODEX, Commission Directive 2006/125/EC and EFSA Scientific opinion [3,4].

[1] EFSA Panel on Dietetic Products, Nutrition and Allergies, EFSA Journal 12(7):3760 (2014) 106pp.

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- [3] WHO-FAO, CCNFSDU CXS 74-1981 (2017).
- [4] European Commission Directive 2006/125/EC, Official Journal of the European Union L 339/16 (2006).

**DETERMINATION OF MULTI-PESTICIDE RESIDUES IN VEGETABLE PRODUCTS USING A “REDUCED-SCALE” QUECHERS METHOD AND FLOW-MODULATED COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY-TRIPLE QUADRUPOLE MASS SPECTROMETRY**

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In this contribution an analytical method for the determination of multi-pesticide residues (88 target analytes) in four vegetable products (tomatoes, cucumbers, sweet red peppers and iceberg lettuce) using a “reduced-scale” QuEChERS (quick, easy, cheap, effective, rugged, and safe) extraction method and flow-modulated comprehensive two-dimensional gas chromatography-triple quadrupole mass spectrometry was performed. In particular, the suitability of flow modulation [with relatively high second-dimension gas flow conditions (8 mL min<sup>-1</sup>)] for trace analyte determination was evaluated.

The samples were prepared according to the QuEChERS procedure as reported by the official European Union method, namely EN 15662:2018, based on the use of 3 g of vegetable product. Matrix-matched calibration processes were carried out for all the samples. The figures-of-merit determined were recovery, linearity, precision, limits of detection (LoDs), and limits of quantification (LoQs). Specifically, recoveries were in the 53-160% range, regression coefficients were between 0.9156 and 0.9999, the LoDs were in the 0.1-6.3 µg kg<sup>-1</sup> range, the LoQs were in the 3.0-21.0 µg kg<sup>-1</sup> range, and coefficients of variation were between 1 and 28% (at the 50 µg kg<sup>-1</sup> level).

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Prot. 2017RHX2E4.



**USE OF SPME-GAS CHROMATOGRAPHY TECHNIQUE FOR THE ANALYSIS OF CHILI PEPPER VOLATILE PROFILE**

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Chili peppers are used as food or spice and are widely used by the food industry as an ingredient for different kinds of flavored and spiced products. The aim of the present study was to determine the volatile compounds of three different species of chili peppers, using solid-phase microextraction (SPME) methods in combination with gas chromatography–mass spectrometry (GC-MS). The detection of marker aroma compounds, in fact, could be used as a parameter to differentiate between species of chili peppers for their detection and traceability in chili pepper food. The sensorial contribution was also investigated to identify the predominant notes in each species and to evaluate how they can influence the overall aroma. Three different pepper species belonging to the *Capsicum* genus were analyzed: *Chinense*, *Annum*, and *Baccatum*. The volatile profile of three extra virgin olive oils (EVOOs) flavored with chili peppers was also investigated. Principal component analysis (PCA) and hierarchical cluster analysis (HCA) of the volatile profiles demonstrated to be a powerful analytical strategy for building a model that highlights the potential of a volatile characterization approach for use in evaluating food traceability and authenticity. The linear retention index (LRI) system was used as a supplementary tool for the recognition of the compounds in combination with the mass spectra, and it made possible the reliable identification and accurate quantification of volatiles in chili peppers and chili-pepper-flavored olive oils [1].

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**SYSTEMATIC STUDY OF BIO-BASED PLASTIC MATERIALS PROPERTIES. PHYSICAL CHEMICAL ASPECTS AND IMMUNOBIOLOGICAL INTERACTION.**

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The growing use of bio-polymers derivatives, directly extracted from natural resources or synthesized from renewable materials poses the increasingly pressing problem of their environmental sustainability. In particular, it should be still ascertained the claimed absence of direct and indirect influence on ecosystems and the health of living organisms, including humans. Although a good number of experimental data are already available, there is still a lack of a systematic point of view, which allows giving certain indications on their environmental impact, in terms of biodegradability and biocompatibility. Among different classes of polymers, we chose those belonging to the class of poly-lactates and poly-hydroxyalkanoates, as there are the most widely used in different applications for replacing plastics derived from fossil fuels. This study aims to contribute to the deepening of this topic through a two-phases experimentation. The first phase involved a systematic investigation of the chemical-physical properties and characteristics of the bio-polymers, utilizing spectroscopic, morphological and thermogravimetric analyses. It has also been evaluated their behaviour in solvents with different polarities to verify their ability to degrade. The second phase was all about the assessment of their effects on natural aquatic environments based on *in vitro* investigation of *Mytilus galloprovincialis* cells.

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**GRAPHENE ENHANCED NEW TECHNOLOGIES FOR REMOVAL OF EMERGING CONTAMINANTS FROM DRINKING WATER**

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The so called “emerging contaminants” (including pharmaceuticals, cosmetics, pesticides and polyfluoroalkyl substances (PFAS)) are increasingly found in tap water [1], calling for the urgent development of new treatment technologies. Graphene based materials have shown great potential for water purification due to their high surface area and their adsorption properties, promoted by multiple interactions pathways with organic molecules and metal ions [2], [3]. Here, we report on the development of new composite membranes based modules enabling ultrafiltration and simultaneous adsorption capabilities in a single step. Polysulfone-graphene oxide hollow fiber membranes (PSU-GO HFs) were prepared and exploited for the removal of ciprofloxacin antibiotic (CIPRO), polyfluoroalkyl substances (PFAS) and heavy metals (Pb, Cu and Cr(III)) [4]. Release of GO nanosheets by the membranes during operation was studied by surface enhanced Raman spectroscopy (SERS) analysis of treated water having the state-of-the-art limit of quantification of 0.1 mg/L for GO nanosheets. Quantification of the removal performances on CIPRO, PFAS and heavy metals were achieved by UV-vis, UPLC-MS/MS and ICP-OES analysis respectively. Removal comparable or even superior than granular activated carbon was observed for contaminants at sensible environmental concentrations.

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**GRAPHENE OXIDE NANOSHEETS AND MICROFILTRATION: A TWO-STEP SET UP FOR WATER PURIFICATION**

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Graphene oxide (GO) and reduced graphene oxide (rGO) nanosheets have shown outstanding adsorption properties, superior respect to other carbon-based nanomaterials, toward several organic [1-2] with a maximum adsorption capacity of about 428 mg/g for methylene blue, a dye and 356 mg/g for ofloxacin, a fluoroquinolone antibiotic.[3] However, the potential as sorbent for water purification is strongly limited by the tedious recovery of the nanosheets after the treatment, which can cause secondary contaminations. Here, we propose a standard, two-step set up based on adsorption and microfiltration that allow the total separation of nanosheets from treated water. A standard procedure for recover of GO and rGO was included, allowing the regeneration and reuse of both graphene nanosheets and the microfiltration module.[4] The procedure was optimized on tap water spiked with ofloxacin or methylene blue and then applied on the removal of mixture of perfluoroalkyl substances (PFAS) from tap water at ppb levels. GO and rGO contamination in microfiltrated water was checked with UV-vis analysis and potability of treated water was confirmed by standard protocols.

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## TARGETED DETERMINATION OF EMERGING CONTAMINANTS BY LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY IN GROUNDWATER AND TREATED WASTEWATER SAMPLES

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Pharmaceuticals and personal care products are a large group of chemicals with diverse structures and physicochemical properties, specifically designed to achieve maximum bioavailability and prolonged duration of action in target organisms. At present, the fate of these chemicals once released to the environment and their health effects of living organisms over the years are still not fully clarified. Therefore, they represent a source of high-concern emerging contaminants whose unintentional presence in the environment requires the development of sensitive and accurate methods for multi-residual determinations at trace levels [1,2].

In the present study, a rapid and selective analytical method is described for the targeted determination of amoxicillin, acetaminophen, metoprolol, carbamazepine, naproxen and diclofenac. A fit-to-purpose high performance liquid chromatographic high resolution mass spectrometric method was developed for the ultra-trace analysis of groundwaters and irrigation waters, obtained by wastewater purification treatments. Full scan MS mode at resolution of 35000 FWHM and Multiple Reaction Monitoring mode provided the optimum conditions for high sensitivity determinations. Six-point linear calibration curves ( $R^2 > 0.9908$ ) were established for all target analytes over a concentration range of 1–500  $\mu\text{g/L}$ . Instrumental detection, calculated by standard solution analysis at a signal-to-noise-ratio of 3, ranged from 0.004–0.27  $\mu\text{g/L}$ . For the sample clean-up, a Solid Phase Extraction procedure was used with a high enrichment factor, leading to method detection limits in water samples in the range 0.015–0.91  $\text{ng/L}$ . Finally, the method was used for the evaluation of the contamination grade by chemicals of emerging concern of groundwaters and irrigation waters, sampled monthly between September 2020 and July 2021. Traces of diclofenac at ppt levels was observed in all the investigated samples over the entire time frame.

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**THE SIMULTANEOUS STABLE ISOTOPE ANALYSIS OF SKIN AND BLOOD GIVES INSIGHTS ON HABITATS SHIFTS IN MEDITERRANEAN LOGGERHEAD TURTLES (*CARETTA CARETTA*, LINNEUS 1758)**

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Marine turtles are key indicator species of ecosystem function and environmental health indicators, however, many of their life-history features remain cryptic. Particularly, the understanding of their year-round feeding habits is critical to establish the effect of contaminants exposure through the marine food web and their adverse health consequences. Here, 24 Mediterranean loggerhead turtles (*Caretta caretta*) with problems related to plastic ingestion were wild-caught alive in the Tyrrhenian Sea (Central Mediterranean), their bloods and epidermis were sampled, and their carbon and nitrogen isotope ratios analyzed. Generally,  $\delta^{13}\text{C}$  values did not correlate between tissues, while  $\delta^{15}\text{N}$  values showed a linear relationship, suggesting more variability in feeding habitats than in trophic levels among turtles. For all animals, the calculated stable isotope niche was wider for skin than for blood, which support a temporal difference in feeding locations. Blood probably indicated the consumption of recent pelagic food, and skin of older more neritic prey. Bayesian mixing models were implemented for turtles sampled in Sicilian waters and results suggested that, generally, prey at low trophic levels were preferred to those at higher trophic positions. Our results support the utility of sampling blood and epidermis from alive and wild animals to investigate simultaneously newer and older ecological features of

sea turtles. Further studies should aim on investigating animals in both good health and with detectable disease issues, to understand how much different threats and healthy status can influence Mediterranean loggerhead turtles' feeding ecology.

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## EVALUATION OF ADSORPTION ABILITY OF AMYLOID PARTICULATES TOWARDS Pb<sup>2+</sup> IONS IN AQUEOUS SOLUTION

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Biocompatibility, easy functionalization and large availability are some of the numerous properties of protein molecules that makes them promising building blocks of biomaterials in micrometer scale [1]. In this context, a particular kind of protein aggregates called amyloids are emerging as promising materials in a large number of applications like biosensors, drug delivery systems, photo-luminescent materials, pollutants adsorbents, etc. [2, - 4]. In this study, Bovine Serum Albumin (BSA) particulates were produced and used as adsorbent material to efficiently remove Pb<sup>2+</sup> ions from polluted waters. Amyloid protein particulates are superstructures with spherical shapes whose radius ranges from few micrometers to hundreds of nanometers with a large surface area and high porosity and were produced through a bottom up approach, heating the aqueous solution of proteins at pH values near their isoelectric point (IP) [5]. The particulates formation was readily controlled and was reproducible as confirmed by results of characterization.

Kinetic and isotherm adsorption experiments were carried out at different experimental conditions changing the pH, the ionic medium and the temperature. The Pb<sup>2+</sup> ions concentration in solutions was measured by means of the differential pulse anodic stripping voltammetry (DP-ASV) technique. Different kinetic and isotherm models were used to fit experimental data. The thermodynamic parameters ( $\Delta G$ ,  $\Delta H$  and  $\Delta S$ ) of adsorption process were calculated by using Gibbs and van't Hoff equations. The reusability of the adsorbent material was verified through column experiments, using EDTA 0.1 mol L<sup>-1</sup> as extracting solution. The highest adsorption was obtained at pH = 5.0, and at  $T = 283.15$  K ( $q_m = 211.3$  mg g<sup>-1</sup>). An adsorption mechanism mainly based on ionic exchange was hypothesized.

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**CHEMICAL-PHYSICAL CHARACTERIZATION OF LEACHATES FROM LANDFILL OF MUNICIPAL SOLID WASTE. STUDY OF THERMODYNAMIC PROPERTIES**

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Landfill leachate is an aqueous matrix that is generated by the percolation of rainwater in the body of a landfill. Leachate can contaminate nearby aquifers due to its content in heavy metals. However, dissolved organic matter (DOM) is also an important component of the leachate, as being made up of humic and fulvic compounds, originating from a series of biological and chemical processes involving the DOM itself; these can act against dissolved metals, as carriers of the same in the aquifers [1]. For the purposes of this study, 7 leachate/water contaminated by leachate samples were collected from a landfill of municipal solid waste according to the guidelines of Italian Legislative Decree 152/2006. Three of these matrixes were collected inside the landfill area and four outside it, downstream. The chemical and physical parameters were analysed according to Table 2 groundwater Part IV Annex 5 Title V of the Italian Legislative Decree 152/2006 and the results obtained showed for some metals and organic compounds, exceedances with respect to the contamination threshold concentrations. The organic and inorganic fractions of the leachate were determined according to procedures reported in the literature [2], from which it was demonstrated that the content of organic and inorganic matter in the various leachate was clearly different, despite having been sampled from the same landfill. The acid-base properties of the leachate were determined by potentiometric titrations at  $T = 298.15$  K, processing the experimental data using the simplified model called Polyprotic-Like model [3], already tested on many classes of high molecular weight polyelectrolytes. On the dry residues obtained at 383.15K and from those obtained after calcination at 923.15K, investigations were carried out by FT-IR, SEM, XRD TGA in order to have more information on the composition of the main elements of the leachate.

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## NANOPLASTICS IN MUSSEL TISSUES: FIRST EVIDENCE, IDENTIFICATION, AND QUANTIFICATION.

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Nanoplastics (NPs), (particles size <1 µm) due to their biological, chemical, and physical features can pass the cell membrane posing serious risks to ecosystems, wildlife, and human health. Quantifying and characterizing NPs is still a big challenge due to methodological limitations. A new highly sensitive technique - thermal desorption proton mass spectrometry (TD-PTR-MS) - already used to estimate NPs presence in remote places of the world (1), was now applied to assess the presence of NPs in mussel tissues. The whole organism was digested following an optimized methodology removing 98% of organic matter (2). Then the digestate was filtered on decreasing pore size filters to separate micro and nanoplastics in size fractions and analyzed by TD-PTR-MS. In this technique, samples are heated for thermally generating plastic vapors, which enter the PTR ionization unit (H<sub>3</sub>O<sup>+</sup>-ions), then the ions are separated by a time-of-flight mass analyzer and detected. The identification is carried out by combining four specific fingerprinting algorithms; a strict detection limit in processing the data was used and only ions with m/z > 100 were considered for fingerprinting (3). For the quality control/assurance, we analyzed three different blanks: system blanks (clean vials); material blanks (clean filters); complete procedural blanks (filters exposed to all the steps of extraction and measurement), samples and blanks spiked with a known amount of polymer standard. Finally, for the first time, NPs were detected, chemically characterized, and quantified in mussel tissues.

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## SAMPLING AND ANALYSIS OF MICROPLASTICS IN ENVIRONMENTAL MATRICES

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In the last decade or so, the number of studies on microplastic (MP) pollution have increased rapidly due to the growing awareness of the potential health risks related to their occurrence [1,2].

The collection and treatment of samples are the most important steps to obtaining an adequate determination of microplastic amount without contamination. According to the type of environmental matrix considered, different sampling techniques are needed. For the water compartment, the main sampling methods are sediment recovery (from the seafloor, beaches, etc), use of marine trawls to collect particles within the water column, and examination of plastic fragments ingested by marine organisms. Regarding atmospheric microplastics, most of the studies have employed a passive sampling of deposited material, while only a few studies have used active sampling, often in comparison with passive ones. Once sampling has been carried out, the analysis of MPs can be done through several procedures such as separation, identification, and quantification. An optical microscope can be used for the identification of microplastics, obtaining images for analysis, which provide the shape and number of the microplastic particles. Although new accurate and sophisticated analytical methods (Pyr-GC-MS and other MS-based methodologies) have been reported in literature, the characterization of samples in terms of polymer composition is mainly performed by FT-IR and Raman spectroscopy.

Based on our investigation of literature data concerning microplastic pollution in the environment, it can be concluded that a standard procedure for sampling, counting and confirming the presence of microplastic is still not present in the literature [3]. Consequently, concentrations are expressed in different units making the comparison between MP abundance values reported in various works difficult or impossible.

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**MICROPLASTICS IN DRINKING WATER: NATIONAL WORKING GROUP AND ANALYTICAL METHODS**

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Microplastics are “emerging” heterogeneous contaminants with a complex toxicological profile. Extremely widespread in water bodies, microplastics have been studied for some time due to the possible effects on human and animal health. Drinking waters could be a potential source of microplastics due to the contamination of surface and ground waters [1]. According to Directive (EU) 2020/2184 [2] on the quality of water intended for human consumption, the European Commission will adopt a methodology to measure microplastics with the aim of including them on the watch-list, in order to respond to growing concerns in a dynamic and flexible way. On this basis, the Joint Research Center (JRC) launched a scientific project in order to harmonize experience and knowledge about microplastic analysis in drinking water, requiring support from the national technical-scientific representatives. So, the Italian National Institute of Health (ISS), with the coordination of the Italian Ministry of Health, has defined [3] a national working group which include experts from the National Research Council (CNR), national and local environmental Authorities (SNPA: ISPRA and ARPA), Universities and Water Suppliers (Utilitalia). This group will work on: (i) JRC and EC support on national expertise about microplastic monitoring in drinking water (ii) development of national analytical method for microplastic in drinking water to be presented to the JRC. The working group will meet for the first time on June 16, 2022 to discuss regulatory aspects, analytical methods for the opening of proceedings.

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**MULTI-ANALYTICAL TOOLS TO INVESTIGATE SOME APULIAN ILLUMINATED PARCHMENTS DATED BETWEEN XVII AND XIX CENTURIES**

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A collection of preciously decorated parchments, dating back to XV-XIX centuries, is preserved at the State Archive in Bari (Italy). These manufactures involve imperial and papal documents, as well as private certificates of important Apulian families. A diagnostic campaign was carried out to disclose the parchments' materials including pigments, binders, and support. Three parchments were investigated by a minimally invasive approach: a *doctoratus privilegium* (17<sup>th</sup> century) and two replicas regarding a *doctoratus privilegium* and a conferment of the title *equus auratus* (both 18<sup>th</sup>-19<sup>th</sup> century). The following pigments were recognized through X-Ray fluorescence (XRF) with fiber optic reflectance spectroscopy (FORS) and  $\mu$ Raman spectroscopy: red (minium or red lead and cinnabar), green (malachite), and blu (smalt). Gold and silver decorations, confirmed by XRF, contribute to the fineness of the documents. Bovine and rabbit collagen binders were assessed by matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) and reversed-phase liquid chromatography with electrospray ionization tandem MS (RPLC-ESI-MS/MS) coupled with a novel sampling strategy based on *in-situ* digestion of proteinaceous materials using a hydrophilic gel loaded with trypsin [1,2]. Additionally, small parchment fragments were subjected to conventional enzymatic digestion and MS analysis, revealing the occurrence of skin sheep as supporting material. The most relevant results will be presented and discussed in this communication.

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**CAPSULE PHASE MICROEXTRACTION OF ORGANOCHLORINE PESTICIDES FROM ENVIRONMENTAL WATER SAMPLES UTILIZING MONOLITHIC SOL-GEL POLYETHYLENE GLYCOL-BASED MEDIA AS A FRONT-END TO GAS CHROMATOGRAPHY-MASS SPECTROMETRY**

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Capsule phase microextraction (CPME) is a recently introduced sample preparation technique that integrates the extraction and stirring mechanism into a single device. CPME has been proved to be a powerful analytical tool for the monitoring of pesticide residues in water samples [1]. Aiming to exploit the features of CPME in environmental analysis, a simple and rapid protocol based on monolithic sol-gel polyethylene glycol microextraction capsules was developed as a front-end to gas chromatography-mass spectrometry (GC-MS). The main chemical and hydrodynamic factors that could potentially affect the performance of the adsorption and desorption step were thoroughly investigated and optimized. Subsequently, the proposed method was validated in terms of linearity, limits of detection (LODs), limits of quantification (LOQs), accuracy and precision. The LOD and LOQ values were 0.01-0.03 ng mL<sup>-1</sup> and 0.02-0.10 ng mL<sup>-1</sup>, respectively. Furthermore, the relative standard deviation values for all analytes were less than 5.3% for intra-day study and less than 9.2% for inter-day study. The green character of the CPME-GC-MS method was evaluated using complexGAPI index [2]. As a proof-of-concept, the herein developed method was successfully employed for the analysis of different environmental water samples.

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**NEW METHOD FOR THE DETERMINATION OF ENDOCRINE DISRUPTING CHEMICALS IN MEDITERRANEAN MUSSEL (MYTILUS GALLOPROVINCIALIS) USING ULTRA-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY**

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In the last decades, anthropogenic activities have led to the discharge of hundreds of pollutants into the environment with the consequent unintended accumulation in marine ecosystems. Many of these pollutants have the potential to mimic natural hormones and are included in the group of chemicals commonly called “endocrine disrupting chemicals” (EDCs).

Bivalves can be used as bioindicators of aquatic pollution because of their abundance, low mobility (sessile organisms), and tendency to bioaccumulate the micro-pollutants in their body (biomagnification). Mussels also play a key role in the trophic chain as they are an important food source for many species.

Most of the available methods applied in such specimens address a limited number of bisphenols or parabens or a single EDC family. On the other hand, current legislation limiting the use of BPA is leading to its replacement with BPA analogues for the production of polycarbonate plastics and epoxy resins, compounds that have not been considered. For this reason, new research that includes this type of chemicals in the proposed methods is required.

In summary, the present work aims to validate an accurate and sensitive UHPLC-MS/MS method for the determination of six antimicrobials / disinfectants (methylparaben, ethylparaben, n-propylparaben, n-butylparaben, phenylparaben, and triclocarban) and seven bisphenols (bisphenol A, AF, B, E, F, P, and S) in *Mytilus galloprovincialis* samples. The method involves a sample treatment based on UAE, and a clean-up based on the use of d-SPE with C<sub>18</sub> sorbent. The proposed methodology was satisfactorily applied to wild samples collected from five different areas of the Mediterranean coast of Granada (southern Spain). The study revealed the EDCs presence in this aquatic species and the usefulness of Mediterranean mussel as a bioindicator of contamination of this type of pollutants.

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**SUPERSTAR - SUSTAINABLE PRESERVATION STRATEGIES FOR STREET ART: AN ITALIAN PROJECT ON THE SAFEGUARD AND PRESERVATION OF STREET ART**

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Street art has been recognized part of our cultural heritage only in the latest years. The ephemeral character, free access, and exposure to the environment and anthropic actions, make public paintings vulnerable to neglect, removal, vandalism, and degradation. Beyond that, the strategies aimed at their preservation and fruition are rather unclear or lacking.

The project PRIN-2020 SUPERSTAR *Sustainable Preservation Strategies for Street Art* (2022-2025) <https://prin2020superstar.dcci.unipi.it/>

sets as a goal the definition of innovative guidelines for the preservation strategy of street art, aimed at safeguarding its powerful social and cultural message in the urban context. The combination of non-invasive and micro-invasive techniques will shed light on the chemical-physical properties and vulnerability aspects of modern paint materials that constitute street artworks. The studies performed in the laboratory on reference materials will be supported by research performed on case studies, focused on the materials used by the artists, the environmental risks and anthropic stress. Relevant case studies have been selected, located in different environmental urban contexts in Milan, Torino and Pisa, in collaboration with municipalities and with urban art curators.

Thanks to the effective collaboration among a wide team of researchers with complementary expertise, involved in the various participating units, and to the interaction with conservation institutions and entities engaged in safeguarding public urban art, the project will contribute to define future preservation strategies. The following outputs are expected: optimized innovative cleaning procedures for the restoration of outdoor murals and for

the removal of vandalistic graffiti; selected protective coating materials with particular attention to durability aspects; and an integrated protocol for long-term sustainable monitoring and conservation.

**DETERMINATION OF MICROPLASTICS IN SEWAGE SLUDGES BY PY-GC-MS**

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Nowadays, environmental contamination by microplastic (MP) is a commonly known concern. Nevertheless, clear statements on its environmental risk and mitigation actions have not been fully established yet. Moreover, standardised analytical methods for MPs quantification are still missing. Wastewaters can collect the contamination deriving from domestic activities, including both primary and secondary MPs as influent to wastewater treatment plants (WWTPs). Synthetic fibres exiting from washing machines are one of the main sources of domestic MPs. Although the removal rate of WWTPs is rather effective, during water depuration processes MPs can be retained in sewage sludges. Sewage sludges can be employed in agriculture as soil conditioners; therefore, they can be an indirect source of MPs contamination of soils. This study aimed at determining the type and amount of MPs in different sewage sludges from a municipal WWTP by pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS). The main objective was to provide information about the main potential source of domestic contamination and clarifying the role of WWTP in MPs reduction. Sewage sludges were dried in oven, milled and sieved (at 0.3 mm). Homogenised samples were oxidised with Fenton’s reagent and filtered on quartz fiber filter (0.3 µm). Filters were folded and directly inserted into pyrolysis cups. Py-GC-MS analyses were performed under thermochemolysis conditions (addition of tetramethylammonium hydroxide) and polymers were quantified by internal standard calibrations. Thermochemolysis was controlled by specific standard. Procedural blanks were developed to consider the possible contamination derived from the method. The contamination from the laboratory was also taken into account by the periodical analysis of air-exposed filter, located at the working stations of each step of the analytical procedure. Due to the lack of a clean matrix, the recovery was calculated on polymers intentionally added to blank procedure.

## MULTI-ANALYTICAL PROCEDURE FOR THE INVESTIGATION OF THREE BYZANTINE SEALS

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During the Roman period and in the Middle Ages, lead was widely used since it was easily extracted from common minerals, such as galena and cerussite, as well as being a by-product of silver production [1].

In this work, three Byzantine lead seals from the archaeological site of San Pietro in Castello (VE) were studied. In the case of lead artefacts buried for many centuries, various coloured compounds can form on the surface, such as red PbO [2] or grey-white PbCO<sub>3</sub> and PbSO<sub>4</sub>. These are stable compounds that do not affect the lead matrix of ancient artefacts, while volatile organic compounds (VOCs) can cause active corrosion of lead [3]. A multi-analytical approach combining non-destructive and micro-invasive techniques was adopted for the analysis of the three seals. First, ED- $\mu$ XRF analysis was performed. The acquired spectra highlighted the presence of Pb, Cu, Sn, Sr and Fe. To identify the compounds that constituted the surface patina of the seals, a few mg of material was taken and analysed by ATR-FTIR. To obtain the composition of the alloy, micro-destructive analyses were required: a few mg of material was taken from each seal and dissolved in HNO<sub>3</sub>. Subsequently, ICP-AES and ICP-MS analyses were carried out to quantify Pb, Sn, Cu, Ag, P, Fe, Sb, S, As, Bi and Zn. This multi-analytical approach allowed us to identify PbSO<sub>4</sub> on the surface and to determine that seals were mainly composed of Pb (>99 wt%), with smaller percentages of Sn, Cu, Ag and Fe. The analyses performed may be preparatory to any possible consolidation and conservative restoration of the lead seals when subject to corrosion/mineralization processes.

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## IS pXRF A SUITABLE ANALYTICAL TECHNIQUE FOR MINING PROSPECTION AND GEOCHEMICAL CHARACTERISATION OF CONTAMINATED SITES?

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Portable X-Ray fluorescence analyzers (pXRF) are widely used for elemental analysis on several matrices in a variety of scientific and industrial fields such as environmental geochemistry, mining, archaeometry and metallurgy. Although its versatility and ready-to-use merits during both field campaigns and in the laboratory, pXRF should be used for quantitative analysis only following a careful validation of accuracy (*via* CRM analyses) or comparability with confirmatory samples (e.g., analysed by means of ICP-AES or ICP-MS). The reference method used to establish data quality includes three levels: definitive > quantitative > qualitative [1].

In this study, several samples collected at two mining district of Friuli Venezia Giulia region (Italy) were analyzed by an Olympus<sup>TM</sup> analyzer (VCA) using both “Soil” and “Geochem” methods. Results were compared with ICP-MS for metal(loid) content and DMA-80<sup>TM</sup> for total Hg determination. Differences of comparability between grounded and ungrounded samples were evaluated to evaluate the efficiency of the pXRF analysis on heterogeneous samples, in order to minimize time-consuming operations.

Results indicate that low concentrations and interferences are the main source of error in pXRF measurements. Samples from the Cu-Sb mine site of the Mt. Avanza reached the quantitative level for the majority of the selected elements, due to relatively elevated concentrations of the analytes and minor levels of interfering elements such as Pb. Conversely, at the Raibl Pb-Zn mining site, the high amounts of Pb (>1%) interfere seriously with As, and natural low-concentrations of e.g., Cr, Cu, Hg and Sb make undetectable such elements due to the geochemical characteristics of the host-rock and the ore minerals. False positives occur in the determination of some elements indicating that spectral analysis is always needed before using the pXRF data.

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## A CLEAN AND SAFE METHOD TO DISPLACE HF AFTER MICROWAVE-ASSISTED ACID DIGESTION

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Microwave-assisted acid digestion is a routine technique for solid sample dissolution for subsequent elemental composition or isotope ratio analysis. As a general procedure, a mixture of pure acids is placed in a polymeric container (e.g., PFA, PTFE) and then treated with microwave radiation to reach high temperature and pressure needed for sample dissolution. The choice of the acid mixture is strongly related to the nature of the sample matrix. As an example, the complete dissolution of siliceous materials (e.g., glass, sediments, soils) requires the forced use of hydrofluoric acid (HF). Although highly efficient in dissolving silicates, this acid exhibits many detrimental effects under the analytical point of view (e.g., formation of insoluble salts, corrosiveness to glassware) that make its removal essential after digestion. The displacement of HF is conventionally carried out by evaporation in open-vessel systems: atmospheric contamination and/or loss of analytes can occur when fuming-off HF owing to the non-ultraclean conditions (chemical hood) necessarily adopted for safety reasons. This feature clearly hinders elemental determination at the ultra-trace level. To overcome this limitation, we have recently developed a clean and safe microwave-assisted procedure to induce the evaporative migration of HF inside a sealed “vessel-inside-vessel” system [1]. This sample holder configuration involves the insertion of a loosely closed PFA container, filled with the acid mixture and the sample to be digested, inside a commercial PTFE vessel containing a few milliliters of a scavenging solution (ultrapure H<sub>2</sub>O and H<sub>3</sub>BO<sub>3</sub> were assessed). We demonstrated that during microwave cycles HF efficiently migrates from the digestion solution to the scavenger via a simple physical mechanism, and then, it can be safely dismissed/recycled. This protocol allows for 99.9% displacement of HF by performing two additional microwave cycles after sample dissolution. The procedure was validated by a siliceous soil reference material (NIST 2710). Importantly, no external or cross-contamination was observed for the 27 trace elements investigated thanks to the properly designed sealed “vessel-inside-vessel” configuration. This protocol is therefore compliant with elemental determination at the ultra-trace level when the utilization of HF is mandatory.

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## VALORISATION OF SEAFOOD PROCESSING WASTE AS BIO-ADSORBENT MATERIAL FOR WATER REMEDIATION

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Environmental pollution caused by heavy metals is a global problem leading to adverse effects on ecosystems, biodiversity, and human health.<sup>[1]</sup>

Among the most common physical and chemical approaches for heavy metals removal from water, adsorption is an effective and economic technique, offering flexibility in the design and operation, and a vast variety of adsorbent materials. The use of natural or waste materials as adsorbents has been largely studied to favour eco-friendly approaches in environmental remediation applications. Among the waste products generated by food industry, mollusc shells have composition and structure characteristics suitable for the removal of heavy metals dissolved in water bodies. Indeed, many studies had reported the capability of molluscan shell powder to adsorb heavy metals from water aqueous matrices.<sup>[2]</sup>

In the present study, oyster shells were used to adsorb metals ions; these materials were characterised and their performances in the removal of metal ions from aqueous solutions were evaluated. To investigate the kinetics and the thermodynamics of the adsorption process, adsorption measurements were carried out at different metal concentrations. Inductively Coupled Plasma - Mass Spectrometer (ICP-MS) was employed to determine metal ions concentration in the solution before and after the contact with the adsorbent materials. Moreover, the adsorbent material was tested as a possible water remediation solution in a local enterprise that cultivates oysters and, consequently produces waste composed of shells, which were used in this study.

The results obtained showed high adsorption capacity of oyster shells towards the metals considered, highlighting a fast adsorption kinetics especially for cadmium. These findings show that mollusc shells are a suitable resource to prepare eco-compatible adsorbent materials for remediation technologies.

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**NANOSTRUCTURED TITANIUM OXIDE EMBEDDED IN ION-EXCHANGE RESINS: A NOVEL SOLID-PHASE EXTRACTION (SPE) MATERIAL FOR GLYPHOSATE ANALYSIS**

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Glyphosate (GLY) is among the most debated and controversial contaminants of our time. Its widespread use as a non-selective broad-spectrum herbicide emphasized the evaluation of the potential negative impact on human health and the environment.[1] Hence, an extensive monitoring of GLY in different environmental matrices at low concentration levels is required for a better understanding of its fate in the ecosystem.[2] In this light, several analytical methods were reported for GLY in past years, mostly based on derivatization prior to SPE and liquid chromatography (LC) analysis, using reverse phase (RP) materials. Recently, good analytical performances were obtained by ion chromatography coupled with high-resolution mass spectrometry, but the system is quite expensive and not available for all laboratories that have to routinely quantify GLY.[3]

Herein, we present a preliminary investigation on nanostructured titanium oxide (TiO<sub>2</sub>) embedded in ion exchange resins as a potential Solid Phase Extraction (SPE) material for pre-concentration of GLY from water and seawater samples. Experimentally, nanosized TiO<sub>2</sub> particles embedded in the crosslinked polymer were synthesized according to Burato et. al. [4] by using a nano casting technique assisted by commercially available polystyrene-divinylbenzene (pS-DVB) Lewatit® K2621 as a template and Titanium (IV) butoxide as the starting material. The synthesis was modified in order to increase the load of TiO<sub>2</sub> and surface area. Physicochemical characteristics of particles were evaluated by FTIR spectroscopy, scanning electron microscopy (SEM), and energy Dispersive X-Ray (EDX).

The ability of the proposed SPE material in concentrating GLY was tested under different conditions of analyte concentration, water sample volume, pH, elution solvent and complexity of the matrix.

The results showed that nano-casted metal oxides are very promising materials to be used as sorbents. In addition, the possibility of synthesizing different metal oxides by changing the metal precursors and ion exchange resin opens up new prospects in the field of adsorbent material as SPE for various emerging contaminants not amenable to be extracted and concentrated with conventional RP sorbents.

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**CONVERSION OF ISOEUGENOL INTO VANILLIN IN ARTIFICIAL SNOW AND ICE.**

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In this work we investigated the (photo)chemistry of isoeugenol, a methoxyphenol often found in biomass-burning-derived aerosols, in both artificial snow and ice. Biomass burning releases semi-volatile compounds (SVOCs) in the atmosphere, which have an atmospheric lifetime that allows their transportation towards distant environments, like alpine glaciers and polar snowpacks. Among these SVOCs, methoxyphenols are an important class, like vanillin, vanillic acid, syringaldehyde, syringic acid, and are often used as environmental tracers of burning biomass in snow/ice (Wan et al., 2019).

Here we focused on the degradation of isoeugenol (50  $\mu\text{M}$  as initial concentration) in artificial snow and laboratory ice at 243 K, under visible light and in the dark. We used Rose Bengal (RB) as photosensitizer for singlet oxygen ( $^1\text{O}_2$ ) under visible light, as carried out in previous works (Bower et al., 2013). Indeed,  $^1\text{O}_2$  has been found to oxidize isoeugenol into vanillin in liquid solution at room temperature (DellaGreca et al., 2007). We observed the conversion of isoeugenol into vanillin by HPLC-UV analysis of irradiated snow samples in the presence of RB. However, we surprisingly found that the same transformation can take place even in the dark in both snow and ice, and in the absence of RB. Therefore, to get insights into the dark oxidation of isoeugenol, we investigated the effects of different salts (NaCl, NaSO<sub>4</sub>, NaClO<sub>4</sub>), pHs and O<sub>2</sub> concentration of air on the vanillin formation in both snow and ice. The results of this study can help to better understand the environmental fate of methoxyphenols in the atmosphere (e.g., in clouds) and in snowpacks.

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**MONITORING EMERGING CONTAMINANTS IN ANTARCTIC SEAWATER: PAST, PRESENT AND FUTURE CHALLENGES**

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The investigation on the presence of contaminants in Antarctica has been included in several research projects within the Italian National Antarctic Research Program (PNRA). The impact of the research station activities, as well as long-range atmospheric transport, can cause classical and emerging contaminants (ECs) to be found in Antarctic seawater [1]. Still, the contamination levels in remote regions are generally so low that classical spot sampling may not provide sufficient sensitivity. Two powerful approaches to overcome this problem are biomonitoring and passive sampling.

Marine organisms can accumulate pollutants during their lives, sadly representing a sort of natural “long-term” passive sampler. The Antarctic Environmental Specimen Bank collects different kind of samples with validated procedures, to permit retrospective studies. In this framework, pooled samples of *Adamussium colbecki*, collected at Terra Nova Bay from 1996 to 2009, were used to monitor the presence of ECs, which, at that time, were unknown or not monitored. A methanol extraction followed by SPE was applied to the *A. colbecki* samples for the subsequent LC-MS/MS analysis of several ECs, including pharmaceuticals, UV-filters, perfluorinated compounds and tracers. Among them, perfluorooctanoic (PFOA) and pefluorooctanesulfonic (PFOS) acids and two UV-filters were detected in all samples, while Triclosan, Bisphenol-A, and some anti-inflammatories were detected at low levels in few samples.

However, the main drawback of biomonitoring is represented by the matrix complexity. The use of passive samplers is a powerful alternative. Polar Organic Chemical Integrative Samplers (POCIS) were deployed in water for few weeks during the latter Antarctic expedition, both in Terra Nova Bay and in the wastewater treatment plant of the Italian research station, enabling contaminant pre-concentration and their following detection by LC-MS/MS. All these data provide a snapshot on the past and present Antarctic contamination by ECs. A systematic study in the next years is desirable to allow long-term considerations.

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**WET WIPES: A STUDY OF THE COMPOSITION, PHOTODEGRADATION, AND RELEASE OF MICROFIBERS BASED ON THERMOANALYTICAL TECHNIQUES**

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Wet wipes are spunlace nonwoven fabrics obtained through the binding of fibers with water jets [1], used to delivery and apply skin-touching personal care products. Wet wipes are commonly composed of cellulose fibers, synthetic fibers, or blends of the two [1]. The intensive usage of wet wipes has increased the concern of the scientific community about microfiber pollution [2,3] and the release of additives in the environment [4].

We investigated different classes of wet wipes in commerce in UK: flushable according to UK certification, biodegradable but not flushable, and conventional. Evolved Gas Analysis-Mass Spectrometry (EGA-MS) allowed us to study the thermal profile of the materials constituting the wet wipes, while their composition and the presence of additives were assessed by multi-shot Pyrolysis-Gas Chromatography-Mass Spectrometry (Py-GC-MS). Moreover, the samples were immersed in distilled water and subjected to artificial photoageing to simulate their behaviour in the environment. The photoaged tissues and the microfibers released in the water were analyzed by Py-GC-MS. All the samples showed significant releases of microfibers and, in the case of the blend composed of cellulose and polypropylene, a tendency to release preferentially cellulose microfibers was observed.

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**CAPSULE PHASE MICROEXTRACTION OF ORGANOCHLORINE PESTICIDES FROM ENVIRONMENTAL WATER SAMPLES UTILIZING MONOLITHIC SOL-GEL POLYETHYLENE GLYCOL-BASED MEDIA AS A FRONT-END TO GAS CHROMATOGRAPHY-MASS SPECTROMETRY**

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Capsule phase microextraction (CPME) is a recently introduced sample preparation technique that integrates the extraction and stirring mechanism into a single device. CPME has been proved to be a powerful analytical tool for the monitoring of pesticide residues in water samples [1]. Aiming to exploit the features of CPME in environmental analysis, a simple and rapid protocol based on monolithic sol-gel polyethylene glycol microextraction capsules was developed as a front-end to gas chromatography-mass spectrometry (GC-MS). The main chemical and hydrodynamic factors that could potentially affect the performance of the adsorption and desorption step were thoroughly investigated and optimized. Subsequently, the proposed method was validated in terms of linearity, limits of detection (LODs), limits of quantification (LOQs), accuracy and precision. The LOD and LOQ values were 0.01-0.03 ng mL<sup>-1</sup> and 0.02-0.10 ng mL<sup>-1</sup>, respectively. Furthermore, the relative standard deviation values for all analytes were less than 5.3% for intra-day study and less than 9.2% for inter-day study. The green character of the CPME-GC-MS method was evaluated using complexGAPI index [2]. As a proof-of-concept, the herein developed method was successfully employed for the analysis of different environmental water samples.



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**INFLUENCE OF THE ANTARCTIC CONVERGENCE ON THE DISTRIBUTION OF FATTY ACIDS ASSOCIATED WITH SURFACE MARINE SUSPENDED PARTICULATE MATTER**

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The study of the lipid component in marine suspended particulate matter (SPM) is crucial for understanding the composition and flows of organic matter in marine ecosystems. The analysis of specific biological targets can play a key role in understanding the transport, transformation, remineralization, and repackaging processes affecting SPM [1]. Since fatty acids (FAs) are the most abundant lipid component in oceanic SPM, they are subjected to numerous investigations, and membrane-derived FAs have been successfully used as environmental biomarkers. The Southern Ocean is an extreme environment for inter-population connectivity as the Antarctic Circumpolar Current represents an unusually strong barrier in the north-south axis owing to its thermal and density-driven isolation of water masses, as well as strong eastward flow [2]. In the present study, the FAs profile of surface marine SPM from the Southern Ocean are determined for the first time, using GC-MS technique. The samples were collected during the crossing from New Zealand to the Ross Sea (near the Italian Station “Mario Zucchelli”), on board the RV Laura Bassi during the 2020 austral summer, approximately every 2 degrees of latitude, to determine the possible influence of the Antarctic Convergence zone on their distribution. Results show an increase in total lipid content from the high latitudes of the Southern Ocean to the Antarctic coastal areas (from a minimum of 0.01 to a maximum of 0.15 mg L<sup>-1</sup>). The distribution of lipid classes shows a greater contribution of polyunsaturated FAs compared to saturated FAs in the coastal areas of the Ross Sea. Moreover, a considerable increase in potential biological biomarkers such as 16:1n7, 18:1n9, 18:2n6, 18:3n3, 20:3n6, 20:5n3 was recorded in the inner Ross Sea.

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**PHTHALATE ESTERS (PAEs) CONCENTRATION PATTERN REFLECTS DIETARY HABITATS ( $\delta^{13}\text{C}$ ) IN BLOOD OF MEDITERRANEAN LOGGERHEAD TURTLES (CARETTA CARETTA)**

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Phthalic acid esters (PAEs) are classified as endocrine disruptors, but it remains unclear if they can enter the marine food-web and result in severe health effects for organisms. Loggerhead turtles (*Caretta caretta*) can be chronically exposed to PAEs by ingesting plastic debris, but no information is available about PAEs levels in blood, and how these concentrations are related to diet during different life stages. This paper investigated, for the first time, six PAEs in blood of 18 wild-caught Mediterranean loggerhead turtles throughout

solid-phase extraction coupled with gas chromatography-ion trap/mass spectrometry. Stable isotope analyses of carbon and nitrogen were also performed to assess the resource use pattern of loggerhead turtles. DEHP (12-63 ng mL<sup>-1</sup>) and DBP (6-57 ng mL<sup>-1</sup>) were the most frequently represented PAEs, followed by DiBP, DMP, DEP and DOP. The total PAEs concentration was highest in three turtles (124-260 ng mL<sup>-1</sup>) whereas three turtles had concentrations below the detection limit. PAEs were clustered in three groups according to concentration in all samples: DEHP in the first group, DBP, DEP, and DiBP in the second group, and DOP and DMP in the third group. The total phthalates concentration did not differ between large-sized (96.3 ± 86.0 ng mL<sup>-1</sup>) and small-sized (67.1 ± 34.2 ng mL<sup>-1</sup>) turtles ( $p < 0.001$ ). However, DMP and DEP were found only in large-sized turtles and DiBP and DBP had higher concentrations in large-sized turtles. On the other hand, DEHP and DOP were found in both small- and large-sized turtles with similar concentrations, i.e. ~ 21.0/32.0 ng mL<sup>-1</sup> and ~ 7.1/9.9 ng mL<sup>-1</sup>, respectively. Winsored robust models indicated that  $\delta^{13}\text{C}$  is a good predictor for DBP and DiBP concentrations (significant Akaike Information criterion weight, AICwt). Our results indicate that blood is a good matrix to evaluate acute exposure to PAEs in marine turtles. Moreover, this approach is revealed as a useful tool to explain the internal dose of PAEs in term of dietary habits ( $\delta^{13}\text{C}$ ), suggesting that all marine species at high trophic levels may be particularly exposed to PAEs, despite their different dietary habitats and levels of exposure [1].

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**FABRIC PHASE SORPTIVE EXTRACTION (FPSE) AS AN EFFICIENT SAMPLE PREPARATION PLATFORM FOR THE EXTRACTION OF ANTIDEPRESSANT DRUGS FROM BIOLOGICAL FLUIDS**

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Antidepressants drugs (ADs) are the most widely prescription drugs to treat major depressive disorder (MDD). The wide increase in the use of antidepressant drugs is due to their role not only in the treatment of major depressive disorder, but also in the management of other related conditions such as anxiety, obsessive compulsive disorder, and nutrition and sleep disorders. Fabric phase sorptive extraction (FPSE), a recently introduced microextraction technique, has been herein applied to achieve a simple and rapid simultaneous extraction of seven common antidepressant drugs (ADs, venlafaxine, citalopram, paroxetine, fluoxetine, sertraline, amitriptyline, and clomipramine) from biological fluids (urine, saliva and whole blood) as well as from post-mortem samples (particularly whole blood and cerebrospinal liquor) collected during autopsies. By eliminating the protein precipitation step and reducing solvent consumption, this technique resulted in sample preparation compliant with Green Analytical Chemistry (GAC) principles. Among all tested FPSE membranes, the sol-gel Carbowax 20M coating on cellulose substrate showed optimal extraction efficiency for ADs. The analysis was carried by high performance liquid chromatography coupled to photo diode array detector (HPLC-PDA). Ammonium acetate buffer and acetonitrile were used as mobile phases. The limit of detection (LOD) ranged from 0.04 to 0.06 µg/mL, whereas limit of quantification (LOQ) was 0.1 µg/mL. The established analytical method has been successfully applied for the bioanalysis of real samples obtained from volunteers as well as samples collected during various autopsies. Unequivocally, this test was the most decisive criterion for evaluating the efficacy of the analytical method and the extraction efficiency of FPSE.

**UNTARGETED LIPIDOMICS BY RP-UHPLC-TIMS-MS REVEALS A RESTRICTED PANEL OF LIPIDS ASSOCIATED TO COVID-19 SEVERITY**

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SARS-CoV-2 is a respiratory virus responsible for causing a prominent metabolic dysregulation affecting multiple organs and generating a broad spectrum of symptoms and clinical manifestations [1]. It becomes essential to pinpoint potential biomarkers able to predict, at early stage, the degree of severity and the possible course of the disease. Lipidomics represents one of the most recent investigative tools able to clarify dysregulations in several molecular pathways and to assist clinicians to evaluate the effectiveness of a pharmacological therapy. For this reason, we investigated lipids alteration in the plasma of Covid-19 patients with different severity (21 controls, 45 mild, 54 severe) at admission time. We developed an accurate RP-UHPLC-TIMS-qTOF method that let us to profile and quantify 348 unique lipids with high confidence. Through a uni/multivariate statistical analysis approach we highlighted a restricted panel of six lipids, including ether-linked lysophosphatidylcholines (LPC-O), lysophosphatidylcholines (LPC) and ether-linked phosphatidylcholines (PC-O), able to discriminate not only between Covid positive and negative subjects, but between mild and severe patients, too. As a result, a machine learning model was built, which was able to predict both the severity (AUC= 0.777, CI: 0.639-0.904) and the outcome (AUC= 0.789, CI: 0.658-0.910) of Covid-19 patients. Lastly, for 25 out of the 99 patients we collected and analyzed a second plasma sample at the change of clinical conditions, and the restricted panel of lipids correctly predicted patients' outcome in 21 cases.

In conclusion, we proposed a novel lipid signature which could be highly useful, in targeted approaches, to predict Covid (+) patients' outcome already at the time of hospitalization.

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**EFFECT OF X-RAY IRRADIATION TREATMENT ON VOLATILE ORGANIC COMPOUNDS (VOCS) OF MOZZARELLA CHEESE**

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Irradiation is a food preservation technology used to inactivate and destroy pathogenic and spoilage microorganisms in order to improve safety and quality of foodstuffs [1-2]. In this study, X-rays irradiation was applied to mozzarella cheese produced with cow milk and a volatolomic approach was used to evaluate the possible modifications on the composition of volatile organic compounds (VOCs), induced by this treatment. Three dose levels, namely 1.0 kGy, 2.0 kGy and 3.0 kGy were investigated and not-irradiated mozzarella cheese samples were used as control (NI). The VOCs analysis was carried out by headspace solid-phase microextraction coupled with gas chromatography-mass spectrometry (HS-SPME-GC-MS). Response Surface Methodology (RSM) and Pareto front, starting from a Central Composite Design (CCD), were used to optimise the SPME extraction conditions in terms of fiber coating, extraction temperature, extraction time and sample amount. A total of 10 classes, including alcohols, aldehydes, alkanes, alkenes, aromatic compounds, carboxylic acids, esters, ketones, methyl esters and oxygen and sulphur-containing compounds were identified. In irradiated cheese samples, hydrocarbons, oxygen-containing compounds, alcohols, aldehydes and ketones increased, due to possible oxidation mechanisms induced by the irradiation treatment. Principal Component Analysis (PCA) and Partial Least Square-Discriminant Analysis (PSL-DA) were used to discriminate the variation of volatile profiles among NI and irradiated samples.

The results highlighted the effectiveness of the volatolomic approach to evaluate the variation of volatile profile with respect to the irradiation, in order to identify potential markers of X-ray treatment.

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**STUDY OF NON-ALCOHOLIC FATTY LIVER DISEASE BY QUANTITATIVE PROTEOMICS AND NETWORK ANALYSES**

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Three advanced quantitative proteomic approaches, based on labelling (SILAC and TMT) or labelling free, in combination with network analyses were applied to a new *in vitro* cellular model of non-alcoholic fatty liver disease (NAFLD) for the first time. This disease is characterized by accumulation of lipids, inflammation, fibrosis and insulin resistance<sup>1</sup>. HepG2, hepatic cells, were used as model and NAFLD was induced by a complex of oleic acid and bovine albumin. The upregulation of Perilipin-2 expression, a protein constitutively present in the vesicles during NAFLD, showed the development of the disease. nLC MS/MS analyses of peptide samples resulted in accurate and reproducible quantitative data of protein fold change expressed in NAFLD versus control cells. The differentially regulated proteins were used to evaluate the involved and statistically enriched pathways<sup>2</sup>. Network analyses resulted in over expression of functional modules in NAFLD, such as inflammation, oxidative stress defence, cell proliferation and ferroptosis. In conclusion the application of advanced bioanalytical approaches in combination with pathway analyses allows the in depth description of NAFLD. This model can be extremely useful in the discovery of new drugs prevent or to cure the disease.

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**SCREENING OF SECONDARY METABOLITES PRODUCED BY FUNGAL STRAINS OF *LASIDIPOLODIA MITIDJANA* ISOLATED FROM *CITRUS SINENSIS***

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The fungal genus *Lasiodiplodia* includes several species with a worldwide distribution and typically associated with plants. A diverse array of bioactive low molecular weight compounds has been described as being produced by *Lasiodiplodia* species<sup>1</sup>. In fact, the capacity to produce secondary metabolites deserves particular attention because these compounds are directly involved in host-fungus interactions.

During the study of the impact of phytopathogenic fungi on citrus trees in Algeria, strains of the novel species *Lasiodiplodia mitidjana* were isolated from necrotic tissues of citrus branch canker<sup>2</sup>. In the present study, a GC-MS based screening of secondary metabolites of four strains of *L. mitidjana* isolated from *Citrus sinensis* was carried out for the first time leading to the identification of several known compounds, such as lasiodiplodin, indole-3-carboxylic acid (ICA), indole-3-acetic acid (IAA), tyrosol, eugenol and succinic acid. Furthermore, the isolation and structural elucidation from the mycelial organic extract of a new spirodioxynaphthalene, namely mitidjospirone, was conducted.

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**GRAFTING NANOMIPS ONTO SURFACES FOR BINDING BIOMIMETIC ASSAYS**

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Molecularly imprinted polymers (MIPs) are synthetic, highly cross-linked biomimetic materials that have cavities capable of selective molecular recognition. They are characterized by the presence of sterically oriented functional groups in such a way as to be complementary to a molecule used as a template in the polymerization process. Recently, molecular imprinting technology has undergone an evolution through the solid phase synthesis of nanoparticles (nanoMIPs). Such innovative approach avoids pitfalls and issues affecting MIPs prepared by more traditional ways of synthesis. Moreover, nanoMIPs have demonstrated to be efficient man-made mimics of natural receptors and a promising alternative to antibodies in sensoristics and bioanalytical applications [1,2].

To date, the application on nanoMIPs in biomimetic binding assays like ELISA or LFIA is hampered by several technical issues among which reproducible and efficient immobilization onto solid surfaces is of high relevance. With the aim to approach this problem, we evaluated the application of nanoMIPs within an immunosorbent assay type platform. Here, nanoMIPs with molecular recognition properties toward the antibiotic ciprofloxacin play the role of recognition elements, so we developed different protocols of immobilization onto the wells of microplate. The immobilization efficacy was observed in combination with the binding capacity of the functionalized surface towards the template labeled with horseradish peroxidase (HRP). In such a way, the HRP signal provides, simultaneously, an estimate of the covalent grafting of nanoMIPs on the well of the microplate and the selective recognition properties towards the template molecule. This work paves the way for the development of efficient and reproducible competitive pseudo-immunochemical assays in which the recognition element is represented by tailor-made nanoMIPs.

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**EVALUATION OF FAECAL BILE SALT HYDROLASE (BSH) ACTIVITY BY NEW BIOLUMINESCENCE CAGED-LUCIFERIN BIOASSAY: GATEKEEPERS ROLE ON HOST-MICROBIOME CROSSTALK IN LIVER-GUT AXIS AND COLON CANCER**

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The gastrointestinal tract hosts a large and complex community of microbes, known as microbiota, that lives in a close relationship with the host and are involved in many physiological processes. One of its important functions is the deconjugation of primary bile acids (BAs) by bile salt hydrolase (BSH) enzymes, where its modulation has been linked to probiotics supplementation. The conjugated BA hydrolysis by BSH produces free BAs that next can be metabolized (i.e. 7-dehydroxylated) to secondary BAs which are involved in different pathologies from IBD to colorectal cancer. BSH-active bacteria modulate the farnesoid X receptor (FXR) signaling which, in turn, is involved in several liver-related diseases, including steatosis, hepatic cancer and non-alcoholic fatty liver disease, linked to altered gut microbiota communities. Therefore, we developed a sensitive bioluminescence-based bioassay for high throughput screening of BSH activity in intestinal content and faeces using the aminoluciferin conjugated to the chenodeoxycholic acid (aLuc-CDCA), that mimics the conjugated physiological glycine CDCA as a substrate for the BSH [1]. aLuc-CDCA doesn't act as a substrate for the luciferase enzyme being “caged” by the amidation and only when released by the BSH activity, induces the BL luciferase/ATP/luciferin reaction. The bioassay has been performed in a black-386 wells microtiter plate where the luciferase was immobilized by physical absorption. 5 µl of 1/10 (v/v) diluted faeces in 0.1 M PBS, pH 7 were used and the aLuc-CDCA ATP 2mM, Mg<sup>2+</sup> 5mM, EDTA 0.1mM added. Then, the kinetics of the light

production were measured with a luminometer. The assay fulfilled all the criteria of accuracy and precision, and the data were also compared with the determination of free CDCA by HPLC-ESI-MS/MS. The complete microbiota will be evaluated by standard microbiology techniques (such as gene sequencing). BSH activity is strongly modified in patients with colon cancer and related diseases and the correlation with microbiota composition will allow studying in more detail the pathological involvement.

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**MS-BASED PROFILING OF HAEMOLYMPH FROM *P. MELAS* (*PTEROSTICHUS MELAS*) EXPOSED TO PENDIMETHALIN HERBICIDE.**

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Pendimethalin is an herbicide, it has been worldwide used for pre-emergence selective control of annual grasses and weeds in croplands. It acts on the polymerization of microtubules interfering with cell division and thus inhibiting the development of roots and shoots in seedlings. However, the persistence of pendimethalin residues in the environment significantly impacts on the soil bacterial and fungal community richness, reducing its growth and affecting the soil biodiversity and fertility. [1]

Carabid beetle *Pterostichus melas italicus* (Coleoptera, Carabidae) was chosen as case of study to investigate the effects of pendimethalin by MS-untargeted profiling of haemolymph. Insect haemolymph plasma is a complex mixture of inorganic ions and cations, amino acids, proteins, lipids, carbohydrates, and their degradation products. It is primarily responsible for supplying nutrients and transferring metabolic wastes to maintain homeostasis. [2] The haemolymph plays a central role in the host immune defense processes. Mass spectrometry has become increasingly valuable for monitoring significant haemolymph molecular changes during developmental stages or infections.

The exposure effect of pendimethalin was tested in vivo on *P. melas* males and females in the reproductive phase of their life cycle to simulate the field exposure in their main period of activity. The MS data were examined with statistical approaches such as principal component analysis (PCA) for possible correlation with the observed biological effects. Moreover, the identity of specific biomarkers was revealed by MS/MS experiments and relevant protein–protein interactions protein association network analysis was performed by the STRING database.

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**EFFICIENT LIPID PROFILE CHARACTERIZATION OF MOZZARELLA CHEESE USING SUPERCRITICAL FLUID EXTRACTION COMBINED WITH LIQUID CHROMATOGRAPHY-HIGH RESOLUTION MASS SPECTROMETRY**

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In Lipidomics, lipid extraction is the first step to isolate a subset of components and to remove any interference. It represents a crucial point for high quality and exhaustive characterization of lipid profile in foods, therefore, it requires continuous in-depth studies [1]. Supercritical fluid extraction (SFE) is an efficient, environmental-friendly and selective alternative to conventional solvent techniques for lipid extraction from food matrices. This technology is based on the characteristic of supercritical fluids to exhibit liquid-like density and gas-like viscosity, allowing better penetration into the solid matrix [2]. In SFE technology, CO<sub>2</sub> is usually employed as supercritical fluid, above the critical temperature of 31 °C and the critical pressure of 74 bars. In this work, an appropriate CO<sub>2</sub>-SFE extraction was used for lipid characterization of mozzarella cheese. The SFE extracts were analysed by means UHPLC-Q-Orbitrap-MS [3] and the MS/MS data analysis were processed by LipidSearch<sup>TM</sup> software to carry out lipid identification. A total of 7 classes of lipids, including ceramides (Cer), diacylglycerols (DG), triacylglycerols (TG), bismethyl phosphatidic acids (BisMePA), cholesterol ester (ChE), lysophosphatidylserine (LPS), phosphatidylethanol (PEt) were measured and the data obtained were appropriately processed by chemometric tools. Finally, a comparison with Folch extraction confirmed that different extraction techniques provide complementary information on lipid composition of foods.

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**PARTICLE-LOADED MEMBRANES AS AN ALTERNATIVE TO TRADITIONAL PACKED-CARTRIDGE SORBENTS FOR DRUG EXTRACTION: IN-DEPTH COMPARATIVE STUDY**

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The performance of particle-loaded membrane, containing 8  $\mu\text{m}$  particles, and traditional packed extraction cartridges, 40  $\mu\text{m}$ , are compared. In this study, solid-phase extraction and ultra-high performance liquid-chromatography (UHPLC) analysis of the drugs, ulifloxacin, fenbufen and felbinac, are duplicated using two devices that contain chemically similar  $\text{C}_8$  bonded silica.

The following parameters are used to assess performance: linearity, recovery, precision, elution volume profile, lowest limit of detection, and loading capacity. Overall, the extraction of ulifloxacin, fenbufen and felbinac from human plasma using particle-loaded membranes achieves better precision, improved concentrating ability, lower detection limits, greater efficiency, and reduced solvent volume requirements compared with traditional packed cartridges.

In addition, elution of drugs from the membrane sorbent is efficiently accomplished using a small volume of mobile phase, eliminating analyte instability problems and the need for additional time-consuming evaporating and reconstitution steps.

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**FAST INTACT GLYCOPEPTIDE ENRICHMENT BASED ON TI-  
IMAC MAGNETIC BEADS**

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N-Glycosylation is one of the most common post-translational modifications (PTMs) of proteins, and plays a critical role in many biological pathways, including cancer development and progression. Serum proteomics for biomarker discovery is hampered by the presence of multiple high-abundance proteins, such as albumin. Glycopeptide enrichment represents an effective procedure to overcome this issue and to identify and quantify low abundance proteins [1].

The aim of this study was to evaluate the performance of different intact glycopeptide enrichment strategies and to test the most effective one on a restricted set of 20 prostate cancer (PCa) and 20 benign prostatic hyperplasia (BPH) serum samples. Five enrichment procedures were compared: one based on the use of non-magnetic microparticles (TiO<sub>2</sub>) and four based on the use of magnetic beads (*Ti-MagReSyn* and *Ti-IMAC HP*). Magnetic approaches have the advantage to be extremely reproducible and potentially automatable and provide high-throughput sample processing.

Relative to the other enrichment methods, *Ti-IMAC HP* provided a higher number of glycopeptides identifications (1198), higher specificity and efficiency (68%). In the set of clinical samples, Ceruloplasmin (CP) and immunoglobulin heavy chain (IGHM) were found significantly increased in PCa sera compared to the BPH group.

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**EXPLORING AND IMPROVING STARCH/GLYCEROL/CMC FILMS PROPERTIES APPLYING MIXTURE & PROCESS DESIGN TO CASTING DEPOSITION**

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The development of biodegradable or biocompatible materials represents an emergent trend in last years and, among all, the field of food packaging is one of the most involved in this trend. In fact, contaminations associated with synthetic food packaging and current increasing concerns related to the negative environmental impact of plastic packaging materials derived from petroleum, have driven significant interest from both academia and industry in natural and biodegradable materials [1].

Most of the papers recently published on this topic have in common the choice of starch, as the main component, glycerol and citric acid, as additives, and casting method, as deposition strategy. Different experimental procedures are proposed, but a rational investigation on the influent factors and an optimisation of reactive amounts and ratios have never been performed. [2]

Starting from the deep literature survey, we identified starch, glycerol and carboxymethyl cellulose (CMC) as the main components and we further expanded our investigation by testing starches from four different sources (wheat, corn, potato and rice) and CMC with three different molecular weight and degree of functionalisation. The very preliminary experiments were focused on defining a suitable experimental procedure, in terms of order of addition of the reagents, the time and temperature required for starch gelatinization, the type of mold and the temperature of the oven.

Then Mixture and Process Design was applied to study the mixture composition, expressed as fraction of starch, glycerol and CMC, the source of starch, the type of CMC and the overall amount of reagents, strongly related to the final film thickness. Once identified model equation and candidate points, D-Optimal Design was employed to select the most informative experiments.

The final aim of this investigation was to define a reliable methodology to produce resistant films, suitable for the application in food thus several qualitative and quantitative responses were investigated, like subjective judgment on the film, physical and mechanical properties.

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## MIXTURE DESIGN FOR RATIONAL INVESTIGATION OF ROCKSALT STRUCTURE FORMATION IN HIGH ENTROPY OXIDES

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Since the path-breaking work of Rosh et al. firstly presenting the possibility to incorporate five different cations into a single-phase oxide system thanks to entropy-related stabilisation,[1] High-Entropy Oxides (HEO) have experienced a steadily increase in popularity due to their unique structural characteristics and correlated possibilities for tailoring of functional properties.[2] Among the various HEO currently under investigation, we focused our attention on systems presenting a rock-salt structure, usually labelled as R-HEO, which are one of the most interesting in terms of material properties and applicability. [1-5]

Starting from the first equimolar mixture of CoO, MgO, NiO, CuO and ZnO, other combinations have been tested in literature but always following a univariate approach, either removing one oxide at the time or testing random combination or amounts of the oxides [1-5]. Having this in our mind, the aim of our work is to rationally investigate and model the formation of rock-salt crystal structure in the overall experimental domain, ranging from pure oxides (CoO, MgO, NiO, CuO and ZnO) up to 5-components mixtures, by a DoE approach. [6-8] To fulfil this aim, we successfully applied D-Optimal Design, to select the most informative training set, Mixture Design to establish the polynomial model relating the response, i.e. the fraction of rock-salt structure in the HEO, to the starting oxides ratio and, finally, D-Optimal Design by addition to select the most valuable test points for model validation.

In the end, we developed and validated a polynomial predictive model that allows to quantify rock-salt structure fraction in the final HEO, relying only on the starting oxides amounts, that will pave the way to a rational and exhaustive investigation of these innovative and interesting materials.

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**A FULL-FACTORIAL DESIGN APPROACH TO SELECT THE OPTIMAL PREPROCESSING STRATEGY FOR MODELING AIMS WITH RAMAN SPECTRA. THE CASE STUDY OF DATTERINO TOMATOES**

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Raman Spectroscopy is used as a non-destructive, fast, and low-cost technique, widely employed for qualitative aims in several fields [1-3].

Before each modeling intent with Raman spectra, it is fundamental to know and explore the spectral pretreatments that may remove specific types of artifacts and highlight the variation of interest [4].

This work, based on a Design of Experiment approach, is planned to systematically evaluate the influence of each preprocessing step and to find an optimal preprocessing strategy.

The technique involves a Full-Factorial Design (FF-DoE) and considers the preprocessing selection as a multi-stage process using the sequential application of smoothing, baseline correction, scatter correction and scaling techniques. The preprocessing steps are applied in a complementary way to assist the achievement of removing the unwanted variation and lead to improved models.

Hereby, a demonstration case is presented by applying the approach to a Raman dataset of datterino tomatoes (traditional and alternative agricultural techniques) for a classification challenge.

The Partial Least Square Discriminant Analysis (PLS-DA) was chosen for the classification analysis of the whole dataset. The dataset was divided into a training set and a validation set. The response variable is the percentage of misclassification in the validation data.

The method suggests that the use of a spectral pretreatment technique chosen by methods often used in spectroscopy, like visual inspection, trials and error or throughout quality parameters, is not sufficient.

The results show that using FF-DoE to select the optimal preprocessing strategy allows for better classification performances with Raman spectra: the samples are all correctly assigned to the right class.

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**IDENTIFICATION OF ILLECIT CONSERVATION TREATMENTS  
IN FRESH FISH BY SPECTROSCOPIC TECHNIQUES AND  
MULTIVARIATE STATISTICS**

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In the field of food control of fresh products, the identification of foods subjected to illicit conservation treatments applied in order to extend the shelf life of the product plays a very important role. Fresh fish products are particularly subjected to this type of fraud, both because of the high commercial value of some fish species, and because they often have to be transported over a long distance from the fishing site, keeping their organoleptic characteristics unaltered. Treatments of this type involve for example the bleaching of the meat and / or the momentary abatement of the microbial load, however, the product continues the degradation process with the production of molecules that can also be dangerous to health.

It is therefore important to find rapid methods that allow the identification of illicit treatments, possibly through technologies that can be applied directly in the field during the control phase.

The study presented here was performed on sea bass samples divided into three groups: controls (stored on ice in the fridge for 3 hours and stored under the same conditions for 24 hours), treated with a solution of hydrogen peroxide and citric acid for 3 hours and treated with the same solution for 24 hours (these last two groups, while they are kept on ice in the fridge). The samples were then characterized through different spectroscopic techniques (micro Raman, FORS, NIR, ATR-IR), and different matrices were characterized: eye, skin, muscle and gills.

The collected data were processed using multivariate statistical techniques of pattern recognition (PCA, cluster analysis) and classification (PLS-DA, SIMCA, Artificial neural networks) in order to correctly identify the samples treated illegally from the control samples.

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**NATURE OR NURTURE: EXPLORING THE DRIVERS OF CHANGE IN THE WORLD'S LARGEST LAKES**

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Over the last half century, many of the world's largest lakes have undergone major modifications in their carbon and nutrient cycles, driven by a combination of changes in land use, population density and climate. One of the key indicators of environmental change is a change in the frequency and intensity in the formation of algal blooms. Using satellite based optical and thermal sensors, available at different spectral, spatial and radiometric resolutions, it is possible to explore changes in the optical properties of the surface waters of the world's lakes and oceans. However, due to the complex optical conditions of these waters and differences in atmosphere conditions, a comparison between dynamics and drivers between lakes has remained a challenge (1). We used high-frequency data, from the MODIS satellite system, combining at sensor reflectance in the visible and near infrared wavelengths and a novel integration of different masking and observational indices, to identify the spatial and temporal distribution of algal blooms in 161 of the world's largest lakes from 2000 to 2020. Overall, there was a significant increase in algal bloom frequency, bloom area, initial bloom date and bloom spatial coverage, particularly evident in lakes at temporal latitudes. We used a random forest regression to highlight potential drivers. Climate related drivers were most linked to initial bloom date, while bloom duration, area, and frequency were more closely associated to increasing economic activities. The use of high frequency algal bloom monitoring provides an important resource for understanding and addressing the challenges in managing these large and fundamental ecosystems, also in the light of international objectives related to the UN Sustainable Development Goal 6.

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**MULTIVARIATE OPTIMISATION OF A HILIC-MS/MS METHOD TO QUANTIFY BOTH POLAR DOPING SUBSTANCES AND LEGAL INGREDIENTS IN DIETARY SUPPLEMENTS**

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Many dietary supplements claim the ability to enhance sports performance and to improve the fitness of the consumers. Athletes can turn to this kind of help, but most of them are supposed to avoid illegal compounds included as dopants in the World Anti-Doping Agency's prohibited list. Occasionally, producers may add illicit compounds without labelling them, leading to unintended doping [1].

Hence, the aim of this study was to develop an analytical method to determine drugs such as diuretics, stimulants and  $\beta_2$ -agonists along with legal ingredients (like caffeine and sweeteners). The selected analytes are very polar ( $\log K_{ow}$  -0.7 – 2.3) and present ionizable functions. Thus, Hydrophilic Interaction Liquid Chromatography (HILIC) was chosen as separation strategy, coupled with tandem Mass Spectrometry (MS), operating in polarity switching mode and multiple reaction monitoring (MRM) to optimise sensitivity.

The instrumental method was optimized using Design of Experiments (DoE). First, a Plackett-Burman (PB) DoE was performed to identify the more influent variables affecting peak areas and resolution. Temperature, water percentage in the eluent, flow rate, as well as type and concentration of buffers as phase modifiers (both formic and acetic acid and ammonium salts) were investigated. A satisfying chromatographic separation was obtained, even resolving theophylline and paraxanthine peaks (isomers presenting the same MRM transition and thus not distinguished by the MS detector). Nevertheless, since the regression models were often not validated by the central point replicates, quadratic models were required. Thus, a D-optimal DoE was set, considering only the most significant variables from PB-DoE results to limit the number of experiments to be performed. Indeed, the buffer choice was quite important for the sensitivity: since anion-forming analytes have lower ionization efficiency and their peak area decreased more with formic acid, acetic acid was chosen (and its amount fixed at 0.1%).

Regarding sample treatment, Homogeneous Liquid-Liquid MicroExtraction (HLLME) is a recent trend. It involves the preparation of a single-phase solution, which allows a theoretical infinite surface area between sample and extraction solvent. Then, an event induces a phase separation and the fraction of interest could be directly analysed. Among HLLMEs, the use of switchable hydrophilicity solvents and acetonitrile extraction via salting-out effect were preliminarily tested on blank, spiked, aqueous matrixes (mimicking those

obtained by the athletes), but further optimisation is still required prior to application to real samples.

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## FINGERPRINTING TECHNIQUE COUPLED WITH QUALITATIVE AND QUANTITATIVE CHEMOMETRIC METHODS FOR THE QUALITY CONTROL OF SAFFRON (*Crocus Sativus L.*)

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This work has the ambitious objective of developing a non-destructive and environmentally friendly method to distinguish genuine saffron samples from the ones adulterated, at various percentages, with the expired spice. Indeed, due to saffron's potential biological and pharmacological properties and its high production costs, this spice is one of the food products most often exposed to fraudulent practices. The blending of cheaper plant-based products resembling *Crocus* stigmas (such as Safflower and Calendula petals or the turmeric powdered rhizomes) proved to be one of the most common adulteration practices of this spice [1]. However, it is not unrealistic that some producers may be adulterating this spice with unsold stocks from previous years. This practice could easily go undetected as the adulterant appears chemically (at least from a qualitative point of view) identical to the high-value product. In detail, 334 samples of Umbrian saffron were analysed by Attenuated Total Reflectance (ATR)- FTIR spectroscopy (**Table 1**).

	Adulteration Levels				Compliant
	100%	40%	25%	10%	Produced in 2020
2016 Adulterant	20 (4)	30 (6)	30 (6)	32 (6)	112 (22)
2018 Adulterant	20 (4)	30 (6)	30 (6)	30 (6)	

**Table 1** Dataset details: number of samples in the training and external validation set (in brackets).

When the analyses were carried out, the samples were compliant (produced in 2020), expired by one year (2018), and expired by three years (2016). The data were exported and elaborated in MATLAB (The Mathworks, Natick, MA; version 2015b) while using in-house functions. Firstly, the most discriminant variables between compliant and expired samples were identified and interpreted. Eventually, both the regression method and the discriminant classification model were applied using the Partial Least Squares (PLS) algorithm [2]. The PLS-Discriminant Analysis model, properly validated, correctly discriminated between compliant and adulterated saffron, and correctly distinguished the samples according to the adulterant (saffron produced in 2016 and 2018). PLS-Regression models, separately built on the two different adulterants, were able to predict the adulteration level with good accuracy.

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**A MULTIVARIATE AND MULTIBLOCK HIGH-THROUGHPUT PROCESSING OF HYPERSPECTRAL DATA**

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Driven by technological advancements, hyperspectral imaging (HSI) systems are today well established in a wide range of analytical chemistry applications. HSI performs rapid acquisitions of full spectral measurements in contiguous spatial portions (pixels) of the surface analysed, providing maps of the distribution of chemical components. The great success of the HSI technologies analysis may be mainly attributed to the possibility of performing measurements in a low-invasive way and – often – without even touching the samples. A critical point, which limited the effective implementation and application of these systems in different analytical sectors, is the complexity of the hyperspectral data processing. Each hyperspectral image, in fact, is constituted by a massive amount of data that requires advanced computational and statistical methods to extract the meaningful (chemical) information.

Nowadays, there is a growing and urgent need for methods and tools that combine advanced computational operations with straightforward graphical interfaces for data organization and visualization. The outputs should be easily readable and interpretable first of all by the users, who aim at digging inside the hyperspectral data, to reveal and discover the composition of the investigated surface, and secondly from the research community, with whom the results are shared. To enhance the complementary information embedded in different spectral data, an efficient multivariate strategy based on the combination of principal component analysis (PCA), brushing, correlation diagrams and maps (within and between spectral blocks) was purposely developed and here presented. The chemometric strategy proved to be highly efficient in data reduction and for the extraction and integration of the most useful information coming from the three different spectroscopies, through an effective data visualization and inter-correlation assessment. As illustrative example, data obtained from the analysis of a multi-layered painting with the first commercial combined XRF and Hyperspectral scanner, were submitted to the chemometric strategy. The analytical instrument, named IRIS and developed by Bruker, allows to simultaneously acquire X-

## CHEMO-PO08

ray fluorescence (XRF, 1-40 keV) data together with visible & near-infrared (VNIR, 380–1100 nm) and short-wave infrared reflectance spectroscopy (SWIR, 1100–2500 nm).

## NON-DESTRUCTIVE AGE ESTIMATION OF BIOLOGICAL FLUID STAINS BY NEAR-INFRARED HYPERSPECTRAL IMAGING AND MULTIVARIATE REGRESSION

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From a criminalistic point of view, the accurate dating of biological traces found at the crime scene, together with its compatibility with the estimated crime perpetration timeframe, enables to limit the number of suspects by assessing their alibis and clarifying the sequence of events. The present study delineates the possibility of dating biological fluids such as semen and urine, as well as blood traces, by using a non-destructive analytical strategy based on hyperspectral imaging in the near infrared region (HSI-NIR), coupled with multivariate regression.

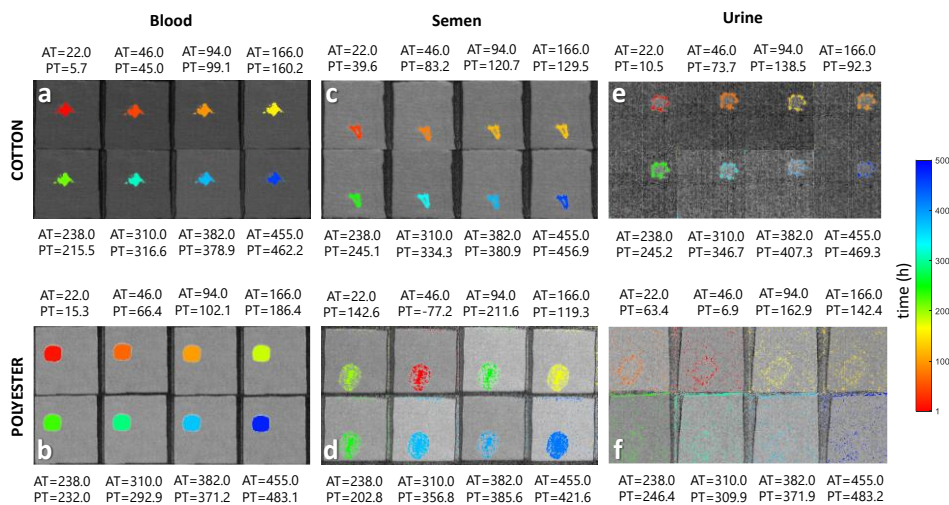


Fig. 1. Color maps related to the different fluids on the two supports. AT = actual time (hours); PT = predicted time (hours) obtained by application of PLS regression.

Investigated aspects of the present study include not only the progressive degradation of the biological trace itself, but also the effects of its interactions with the support on which it is absorbed, in particular the hydrophilic vs. hydrophobic character of fabric tissues (Fig. 1). Results are critically discussed, highlighting potential and limitations of the proposed approach for a practical implementation.

**DOE-BASED EVALUATION AND OPTIMISATION OF AC16 HUMAN CARDIOMYOCYTES STRESS INDUCED BY GLUCOSE APPLYING ANOVA-SIMULTANEOUS COMPONENT ANALYSIS (ASCA) AND LOW-LEVEL FUSION**

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High glucose (HG) condition mediates cardiovascular complications, and oxidative stress, which is implicated in the pathogenesis of diabetic patients.<sup>1</sup> Different metabolic pathways have been proposed to be involved in HG stress, and novel approaches are needed to find potential pharmacological targets. In order to study the biochemical variations that occur after HG treatment in AC16 cells, we have here performed a multi-omics strategy. Their potential resides in the identification of biomarkers for different diseases for both diagnostic and therapeutic purposes. However, the application of multi-omics approaches to a complex biological system requests multivariate approaches and a preliminary study regarding parameters optimisation and variable screening. This study is focused on this preliminary step. A full factorial experimental design (DOE)<sup>2</sup> has been defined to optimise the experimental settings. Two factors have been studied (time and glucose quantity) at different levels: 2 days and 7 days for time and 10, 20, 30 mM for glucose concentration. The high-dimensional nature of the response needs a multivariate approach to analyse the designed data. Here, ANOVA-Simultaneous Component Analysis (ASCA) is proposed to evaluate the effect of the design terms (controlled factors and their interaction) for individual lipidomics, metabolomics and proteomics data set as well as their low-level fusion. ASCA allowed identifying the factors that significantly affect the defined experiments and a selection of the more relevant variables that affect the system. A successive pathway analysis could drive the hypotheses for a possible mechanism.

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**ASSESSMENT AND COMPARISON OF AIR POLLUTANT PROFILES BEFORE AND DURING COVID-19 LOCKDOWN IN FRIULI VENEZIA GIULIA REGION (ITALY) BY USE OF SELF-ORGANIZING MAP AND K-MEANS CLUSTERING ALGORITHMS**

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The spread of COVID-19 pandemic has forced the governments worldwide to subject the population to recurrent lockdown periods. During this period several commercial/industrial activities were closed as well as schools and universities. People was allowed to leave their homes for essential needs. A secondary effect of these restrictions has been a relevant traffic reduction [1]. In Italy the strictest lockdown was during the period March-April 2020. In this study we present the profile analysis of pollutant concentrations recorded in two main cities of Friuli Venezia-Giulia region, namely Trieste and Udine, during the lockdown period in comparison with the same period in 2019.

The data were recorded at two air quality stations classified as “urban background” by the regional environmental protection agency (ARPA-FVG). The data is a collection of hourly means of the following pollutants: PM<sub>10</sub>, PM<sub>2.5</sub>, NO<sub>2</sub>, NO<sub>x</sub>, benzene and toluene. For each site and each year an unsupervised model has been built by use of Self-Organizing Map and k-means clustering algorithms [2,3]. We obtained five profiles for each site that represent the main recurrent “air types” recorded at the stations. Observing the values obtained for each variable and the frequency of each “air type” we classified them as “background air”, “residential heating source”, “low traffic source”, “high traffic source”, “mixed source” (residential heating+low traffic). We could extract the following information: (i) the traffic source profiles were similar in both 2019 and 2020 but less recurrent during the latter for both sites; (ii) the residential heating source (mainly characterized by particulate matter) had similar profile and frequency in 2019 and 2020 for both sites. This is in accord with results presented in already published papers [1,4]. Moreover we could detect a number of outlier profiles that were related (a) with an Asian dust spread event; (b) with differences due to local events/meteorology related to the city position (Trieste by the sea, Udine in the inland).

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## IMAGE ANALYSIS IN FOOD MONITORING

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Imaging, either digital (RGB), multispectral and hyperspectral is becoming more and more applied in food analysis. As a matter of fact, several advantages can be envisaged: color features and chemical composition can be simultaneously taken in consideration; ingredients distribution, hence homogeneity, can be studied; cameras can be implemented on-line and(or in situ and allow real-time monitoring.

Thus, there has been a huge development in chemometrics to handle imaging data. In this work, as a flavour of the different possibilities and challenges three different examples are presented: one concern the industrial pesto production, where a vision system monitor on-line basil quality. For this case of study image segmentation and object detection are relevant aspects; a second one concern defect detection on food surface either monitored by RGB or multispectral camera and where wavelet enhanced multivariate image analysis is employed [1]; finally, a third one concern studying the process of bread staling [2] acquiring near infrared hyperspectral images of bread slice at different times. Here a novel approach, recently proposed by some of us [3] to deal with situation where the spatial spectral interplay is relevant has been applied.

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**OPTIMIZATION OF A SPME-ARROW-GC/MS METHOD FOR DETERMINATION OF VOLATILE ORGANIC PROFILE FROM BREAD SAMPLES.**

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The flavour of a food product is a crucial aspect of its quality, and it can be influenced by raw materials and/or conditions in the manufacturing process as well. The flavour of a food product is usually assessed by expert panel test, however, even if the evaluation provided by the panellists are an important parameter for product quality assessment, objective analytical tools can provide a notable support. Also, they could allow establishing a link among sensory attributes and chemical composition.

In order to characterize the food flavour pattern, many analytical methods have been developed, such as the essential oil extraction with solvents and the collection of the released volatile molecules by means of different sampling techniques. Actually, most methods are based on the direct collection of the headspace, or the trapping of the volatile molecules by Solid-Phase Micro Extraction (SPME) or by Head-Space Sorptive Extraction (HSSE) and gas chromatography (GC) to separate the collected molecules, coupled with mass spectrometry (MS), to have an identification or, just to FID if identification is not of main concern. Solid phase microextraction (SPME)-Arrow is a new green chemistry extraction technology recently employed in the analysis of volatiles in food materials. This technology is based on a novel SPME geometry that can be used to “hunt” target molecules in complex matrices.

The aim of the presented study is twofold:

- i) to develop and validate SPME-Arrow sampling technique coupled with GC-MS instrument for the analysis of volatile organic profile of industrial bread samples;
- ii) to investigate if and how the different process parameters and/or the recipe could have a direct impact on qualitative and quantitative composition of the aroma compounds.

To optimize the SPME-arrow extraction conditions, a strategy based on a rational design of experiments (DoE) [2] was developed. Moreover, an untargeted approach through direct multivariate analysis of GC-signals has been carried out.

A precise knowledge of aroma and of the parameters that influence its variation is of paramount importance for the baking industry, to tune the modifications of the recipe and the production parameters.



## CHEMO-PO13

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**SENSING AND HYPERSPECTRAL IMAGING SELF ORGANIZED  
MAPS: DETECTING ALTERED AREAS IN PAINTINGS**

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Spectral imaging modalities, including IR reflectance, extended UV-VIS, and X-ray fluorescence, play an important role in conservation science. In reflectance hyperspectral imaging, the data are classified into areas having similar spectra and turned into labeled pigment maps, degraded or previously restored areas using spectral features and fusing with other information. Direct classification and labeling remain challenging because many paints are intimate pigment mixtures that require a non-linear unmixing model for a robust solution.

A Self Organizing Map (SOM) [1] is an unsupervised machine learning technique used to produce a multi-dimensional data set while preserving the topological structure of the data. It could be useful to analyze overlapped multispectral images and represent them as clusters of observations with similar values for the input variables. These clusters then could be visualized as a two-dimensional "map" such that observations in proximal clusters have more similar values than observations in distal clusters. This can make high-dimensional data much easier to visualize and analyze [2].

In our work, SOMs have been successfully applied to a multispectral dataset of paintings in order to obtain a best modeling of areas that show non-linear conditions in remote sensing without a pre-existing training dataset. For paintings, in fact, existing spectral databases are small and do not encompass the diversity encountered. Given that painting practices are relatively consistent within schools of artistic practices, we tested the suitability of using SOM on reflectance spectra from a subgroup of well-characterized and standardized paintings in collaboration with a hyperspectral cameras manufacturer. The labeled pigment maps produced were found to be robust within similar styles of paintings. In a subsequent step the output map can be used as training dataset for a Topological Quantum Neural Network-based graph [3].

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**ANALYTICAL AND MULTIVARIATE STATISTICAL APPROACH TO ASSESS HYDROGEOCHEMICAL DIFFERENT ORIGIN OF GROUNDWATER SPRING SOURCES.**

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Groundwater chemistry is the result of extensive, long-term interactions with the bedrock. The chemical composition of the groundwater is controlled by many factors including precipitation, mineralogy of geological horizons and aquifers, climate, and topography. These factors combine to create different types of water [1]. The purpose of this study, conducted in collaboration with the water springs manager, is to establish relationship between the geological information and the chemical composition that enable to differentiate a group of groundwater springs which reside in a very restricted geographical area while their collection occurs along various underground paths conferring different chemical profile.

Partial Least Square Discriminant Analysis (PLS-DA) is a supervised multivariate statistical method that can be employed for the groundwater characterization and classification. Fundamentally, the PLS-DA predictive modelling encompasses PLS component construction (i.e., dimension reduction), and prediction model construction (i.e., discriminant analysis). this method can effectively solve the problem of multicollinearity between multiple variables with a high degree of correlation between them. [2]

Water samples were analysed by two independent laboratory using different analytical method (laboratory 1 and 2) and the manager's analysis laboratory (Laboratory B) proceeded to analyse the water springs over the course of a calendar year monthly. The concentration of main ions ( $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{HCO}_3^-$ ,  $\text{SO}_4^{2-}$ ,  $\text{Cl}^-$ ,  $\text{NO}_3^-$  and  $\text{F}^-$ ) and pH were determined. The consistency of the analytical data was checked by determining the ionic charge balance of the samples. In addition to the chemical features, six hydrogeological (A-F) parameters were used for better discerning water groups related to aquifer lithology of the main reservoir crossed by each water sample [3]

External validation found that the model had good stability and discrimination. Calcium, bicarbonate ( $\text{HCO}_3^-$ ), sulphate, hydrogeological parameter A and B were the most important compositions markers that affected the discrimination results Selectivity ratio for the Projection (VIP) scores. Variables were selected using the VIP score and selectivity ratio for the projection (SR). SR method was found to be more reliable for variable selection purposes.

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**RARE EARTH ELEMENTS (REE) IN GROUNDWATER IN THE ARCHEOLOGICAL SITE OF CUMAE (PHLEGRAEAN FIELDS, SOUTHERN ITALY).**

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The rare-earth elements (REE) and combined yttrium (YREE) elements are ubiquitous with stable and long-lived isotopes; they have been used to trace some geological processes as weathering processes and water-rock interaction<sup>1</sup>. REE were determined on groundwaters collected in the Cumae archaeological site, which is located in the coastal north-western sector of the volcanic district of Phlegraean Fields (Southern Italy)<sup>2</sup>. The results show that the La, Ce, Pr and Nd of REEs are significant in groundwater, and the  $\Sigma$ REE contents in groundwater range from 200 to 2000 ng/L. The abundance of REEs decreases with increasing atomic number (the concentrations of light rare-earth elements (LREEs) are higher than that of the heavy rare-earth elements (HREEs)). Measurements carried out on samples of groundwater collected at different depths (up to 60 m) reveal an increase in the concentration of the HREE elements. The vertical profiles of the REEs are similar for all. Neighboring REEs (e.g., Pr vs. Nd) show very tight correlations.

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## CARBONATE COMPLEXATION OF RARE EARTH ELEMENTS IN THE ALKALINE LEACHING PROCESS OF MINE TAILINGS

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In the last few years, technological innovations have resulted in multiple applications for the use of Rare Earth Elements (REEs) as magnets, battery alloys, catalysts, glass, polishing, and ceramics (polishing compounds, coloring and decoloring agents in glass, stabilizers in ceramics) [1].

REEs are a group of elements (lanthanides plus scandium and yttrium), which have similar physical properties and are often found in the same ore deposits. Today, REE-bearing-carbonates (bastnäsite, REE(CO<sub>3</sub>)F) and phosphates (monazite, REE(PO)<sub>4</sub> and xenotime (Y,Yb)(PO<sub>4</sub>)) are the main rare earth minerals of commercial importance [2].

Italy does not own primary economic REEs deposits so far, even though several small concentrations have been observed in different host rock types, and an interesting occurrence of REEs (also including Y) from the Silius hydrothermal vein system in SE Sardinia (Italy), which was exploited until 2006 for its CaF<sub>2</sub> and Pb resource, is reported [3].

Quantitative extraction of REEs from ores can be performed with well-standardized procedures [2], however, leaching of REEs from fluorite, calcite, and dolomite, which are the main ore minerals extracted at Silius, may require a different strategy [4], due to the risk to have high acid consumption. In this work a new procedure for the recovery of REEs from fluorite-barite-galena ores with calcite gangue from Silius mine is presented.

REEs fluorocarbonates are extracted using, as the leaching solution, 50% K<sub>2</sub>CO<sub>3</sub> at 100 °C.



Moreover, at laboratory scale, this alkaline leaching process allows the recovery of the CO<sub>2</sub> produced as K<sub>2</sub>CO<sub>3</sub> from concentrated KOH, in accordance with a circular flow [5]. Further work is ongoing to scale up the process into a pilot plant, to prove that the method developed within this

research can be economically feasible, socially suitable, and environmentally respectful.

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**RADON LEVELS AND GAMMA RADIONUCLIDES IN GROUNDWATER, SOIL AND AIR IN THE ARCHEOLOGICAL SITE OF CUMAE (PHLEGRAEAN FIELDS, SOUTHERN ITALY).**

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Radon, <sup>222</sup>Rn, originates from the decay of radium, <sup>226</sup>Ra, a member of the uranium (<sup>238</sup>U) decay series. Although radium occurs in virtually all type of rocks and soil, its concentration varies with the specific site<sup>1</sup>. Radon concentrations were measured in soil, air and groundwater in the Cumae archaeological site, which is located in the coastal north-western sector of the volcanic district of Phlegraean Fields (Southern Italy). Radon concentrations were found to vary from 1 kBq/m<sup>3</sup> to 70 kBq/m<sup>3</sup> in soil, 5 Bq/L to 70 Bq/L in groundwater and 20 Bq/m<sup>3</sup> to 50 Bq/m<sup>3</sup> in air. Measurements made at different depths (up to 60 m) show an increase in radon concentration in groundwaters, from 5.0 Bq/L to 120 Bq/L. A positive correlation was observed between the concentration of primordial radionuclides, as <sup>40</sup>K, in the soil and the corresponding concentration in the water samples, indicating the possible leaching of radionuclides from the soil to groundwater.

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## RESIDUES OF THE HERBICIDE: AMINOMETHYLPHOSPHONIC ACID METABOLITE OF THE GLYPHOSATE

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The aminomethylphosphonic acid (AMPA) is the widely occurring degradation product of glyphosate [1]. Massive use of the precursor compounds of the AMPA conducts to the ubiquity of this ligand in the environment and in water, in fact in agricultural soils, AMPA is distributed in the topsoil [2], from here the particles moves towards the waterways by means the rain fall.

There is a lack of information data on the chemical behaviour in water of this ligand and this is essential to better understand its toxicological effects; therefore, it is necessary to know the thermodynamic parameters and speciation of AMPA in aqueous solutions.

The purpose of this communication is to show the chelating and sequestering ability of AMPA towards bi- and trivalent metal cations, such as  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Cu}^{2+}$  and  $\text{Al}^{3+}$ , in aqueous solutions of NaCl, major components of the natural water. The investigations on the acid-base properties and complexing ability of the ligand were performed, by means of potentiometry, while the insoluble species formed during the speciation studies of the  $\text{M}^{n+}/\text{AMPA}^{2-}$  systems, was characterized by means of X-Ray Diffractometry (XRD), Scanning Electron Microscopy (SEM) and InfraRed Attenuated Total Reflection spectroscopy (IR-ATR).

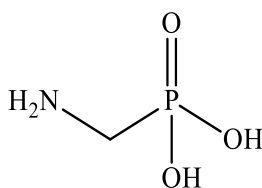


Fig. 1. Structures of aminomethylphosphonic acid (AMPA).

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**MULTI-TECHNIQUE APPROACH TO THE STUDY OF THE CHEMICAL SPECIATION AND THE COORDINATION PROPERTIES OF METALLOPHORES**

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Chemical speciation calculations based on equilibrium data (e.g., stability constants and other thermodynamic parameters) are of utmost importance in several technologically, environmentally and biologically relevant processes.

Potentiometry and UV/Vis spectrophotometry, followed by computer data analysis, are the most widely used techniques in chemical speciation studies, and they still remain among the most adequate and accurate for the determination of the stability constants in solution. Nevertheless, the investigation of more and more complex systems (e.g., ligands with several and different binding sites, very strong chelants, unconventional conditions, multicomponent solutions, etc.) opened up new challenges and questions for solution chemists. As such, other techniques and/or approaches are becoming ever more necessary to get further information, for example, on the nature of species effectively formed, on their structure, and on their reactivity.

These aspects are particularly relevant in the case of chemical speciation studies of metallophores, which are, generally, low/medium molecular weight molecules biosynthesized by living organisms (e.g., bacteria and other microorganisms, insects, plants) to act as very strong chelants towards many metal cations to favor their uptake and/or to lower their toxicity.

In this communication, some examples will be reported to evidence how these techniques and approaches have been exploited to complement potentiometric and/or spectrophotometric results to solve some issues related to the assessment of the chemical speciation and the coordination properties of some natural metallophores towards biologically relevant elements.

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## THERMODYNAMIC SOLUTION PROPERTIES OF ACONITIC ACID AND ITS SEQUESTERING ABILITY TOWARD DIFFERENT METAL CATIONS.

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Trans-Aconitic acid (TA, propene-1,2,3-tricarboxylic acid) is an unsaturated organic acid and mainly found in natural sources like cane molasses [1]. TA is an intermediate of the tricarboxylic acid cycle that is synthesized by aconitase. It has different applications as bio-based plasticizer, acidulant in food processing industry but also as nematicidal and anti-edematogenic activities and for this reason it has attracted attention of researchers worldwide, and for the United States Department of Energy TA is one of the top 30 potential building block candidates [2]. Despite its importance, few thermodynamic data are present in literature, and then new data can be useful to exploit the ability of this “green” molecule. In particular we focused our attention on the determination of the protonation constants of TA by potentiometry in different salt media, namely, NaCl<sub>(aq)</sub>, (C<sub>2</sub>H<sub>5</sub>)<sub>4</sub>NI<sub>(aq)</sub>, KCl<sub>(aq)</sub> at different conditions of temperatures ( $15 \leq t \leq 45$  °C) and ionic strengths ( $0.1 \leq I \leq 1$  mol L<sup>-1</sup>). The interaction of TA with some metal cations (*e.g.*, Ca<sup>2+</sup>, Mg<sup>2+</sup>, Zn<sup>2+</sup>, Pb<sup>2+</sup>, Cd<sup>2+</sup>, Cu<sup>2+</sup>, Mn<sup>2+</sup>, Co<sup>2+</sup>) was investigated as well at 298.15 K in KCl<sub>(aq)</sub> in the ionic strength range  $0.1 \leq I/\text{mol dm}^{-3} \leq 1.0$ . The values of protonation constants at infinite dilution and at  $T = 298.15$  K are:  $\log K_1^H = 6.315 \pm 0.005$ ,  $\log K_2^H = 4.396 \pm 0.005$ , and  $\log K_3^H = 2.949 \pm 0.008$ . In the presence of a background electrolyte, all 3  $\log K^H$  values followed the trend (C<sub>2</sub>H<sub>5</sub>)<sub>4</sub>NI  $\geq$  KCl  $\geq$  NaCl. The differences in the values of protonation constants among the three ionic media were interpreted in terms of variation of activity coefficients and formation of weak complexes. In addition, as regards the interactions of metals with TA, for most of them the main species were the ML, MHL, and MH<sub>2</sub>L. The whole set of the data collected may be crucial for instance, for the development of TA-based materials for natural fluids selective decontamination from heavy metals.

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**REMOVAL OF THE SYNTHETIC AZO PIGMENT NAPHTHOL RED (PR170) BY ADSORPTION ONTO SILICA (SiO<sub>2</sub>) FROM AQUEOUS SOLUTIONS**

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Synthetic dyes are extensively used in many industrial fields, such as textile, paper, leather tanning, food processing, plastics, cosmetics and printing industries. Synthetic dyes are complex organic molecules resistant to many agents (e.g., detergents) and exhibit a considerable structural diversity. Among them, most of the dyes currently used in the industry are azo derivatives and their wastes are toxic and carcinogenic posing a serious hazard to aquatic living organisms<sup>1</sup>. The adsorption of dyes from aqueous solution has proven to be an excellent approach to treat effluents as well as an economical method for dye removal<sup>2</sup>. Naphthol Red (PR170) is a very important type of azo pigment mainly applied in fields like coatings and inks, but no adsorption studies were carried out to develop a strategy to reduce the discharge of this dye in the effluents.

In the present work, the removal of Naphthol Red by adsorption on silica (SiO<sub>2</sub>) was investigated. In particular, the effects of the pH of the solution, the particle size (granulometry) and the pore size (porosity) of the adsorbent on the process were in batch evaluated.

Due to the low water solubility of Naphthol Red, the suspensions were prepared in a water/dimethylformamide (10% v/v) mixture, in an ionic medium 0.1 M NaCl.

Our findings show that the adsorption on silica removes 95% of the pigment from aqueous solutions (pH = 7.00, C<sub>PR170</sub> = 4.00 mg L<sup>-1</sup>).

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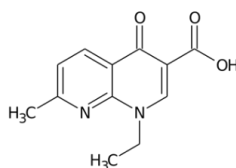
## STUDY ON NALIDIX ACID: ACID-BASE PROPERTIES AND INTERACTIONS WITH BIOLOGICALLY RELEVANT METAL CATIONS

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Among the quinolones, nalidixic acid (NAL, in Figure 1), is one of the most widely used broad-spectrum antimicrobials for the treatment of various infectious diseases. Recently, it has been shown that the interaction between NAL and various metal cations usually present in biological fluids, has considerably increased the antimicrobial activity against Gram-positive and Gram-negative bacteria<sup>1</sup>. Therefore, the determination of thermodynamic formation parameters is crucial to better understand the extent of the interaction between NAL and metal cations and to obtain a speciation model describing its behavior in solution. A preliminary acid-base study was carried out on NAL solutions by potentiometric titrations at three different temperatures (15, 25 and 37°C) and ionic strengths (0.15, 0.5, 1 mol L<sup>-1</sup>). The interaction of NAL with Zn(II), Cu(II) and Mn(II) was subsequently studied by potentiometric titrations in NaCl aqueous solution under different ionic strengths (0.15, 0.5, 1 mol L<sup>-1</sup>) and temperatures (15, 25 and 37°C). By the dependence of the formation constants on the temperature, the enthalpy change values were determined. The sequestering ability of NAL towards the metal cations under study was also evaluated under different conditions. In addition, spectrophotometric titrations were performed to confirm the potentiometric results and to get information on the spectral behavior of the ligand. Valuable information about molecular structures of the complexes were also obtained by mass spectrometry analysis.



**Figure 1.** 1-Ethyl-7-methyl-4-oxo-[1,8]naphthyridine-3-carboxylic acid (NAL).

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**SYNTHESIS OF A NOVEL SUCCINIC ACID-FUNCTIONALIZED CELLULOSE AND ITS SPECIATION MODEL IN THE PRESENCE OF Cd(II) IONS**

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Recently, the demand for new renewable materials to be employed in remediation has grown exponentially. Furthermore, despite the availability of many chelating agents able to efficiently bind metal cations in solution, only few of them are actually suitable to be used to treat metal-polluted wastes. The non-biodegradable EDTA, for instance, ended up being overused, thus becoming a polluting agent itself. In this context, this research is focused on the development of a novel ecofriendly material with potential application in all fields in which metal ion sequestration is desirable. The core idea of biocompatibility lead to the design and synthesis of a novel cellulose derivative (*CS*) functionalized with succinic acid (*SA*). Results from NMR, qNMR and FT-IR ATR experiments evidenced the successful substitution of the succinic acid on the microcrystalline cellulose backbone, with an estimated substitution rate of one *SA* for every six glucose units. The resulting material was investigated in aqueous solution to determine its acidic constants, sorption capability and establish a reliable speciation model in the presence of  $\text{Cd}^{2+}$ . The acid-base properties and the *CS* binding constants towards  $\text{Cd}^{2+}$  were checked by potentiometry in  $\text{NaCl}_{(\text{aq})}$  ( $0.1 \leq I / \text{mol dm}^{-3} \leq 1.0$ ) at  $T = 298.15 \text{ K}$  in a pH range between 3 and 11. Two species (*CS*) $\text{Cd}(\text{OH})$  ( $\log \beta = -5.8$ ) and (*CS*) $\text{Cd}(\text{OH})_2$  ( $\log \beta = -13.41$ ) are formed. Sorption tests were performed by means of kinetic and batch experiments and the residual  $\text{Cd}^{2+}$  concentration in solution was measured by differential pulse anodic stripping voltammetry. In conclusion, *CS* appears to be promising as a tool for heavy metals removal.

**ON SOLUTION EQUILIBRIA OF Ag(I) - ANTHRACENYL  
BISCARBENE BINDING TO BIOSUBSTRATES: SMALL  
STRUCTURE CHANGES PRODUCE DIFFERENT  
THERMODYNAMICS**

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Silver compounds have been tested for many years as antimicrobial, starting from the spread use of silver nitrate in ancient times [1,2]. Among them, Ag(I) complexes of N-heterocyclic carbenes (NHCs) have been extensively used for their diverse applications, like intermediate for transmetallation reactions [3], catalysts [4], and the interest not only as antibacterial agents but also for their application as anticancer drugs increased [5].

Here we propose two Ag(I)-bisNHC (Figure 1) that only differ in the length of the carbon chain attached to the N atom of the imidazole. A study on the thermodynamics of the binding with CT-DNA, DNA G-quadruplex (Tel-23) and bovine serum albumin has been performed. Spectrophotometric and spectrofluorimetric titrations have been used to evaluate the binding constants at different temperatures, and HR-MS experiments enabled us to highlight the different binding modes with the protein.

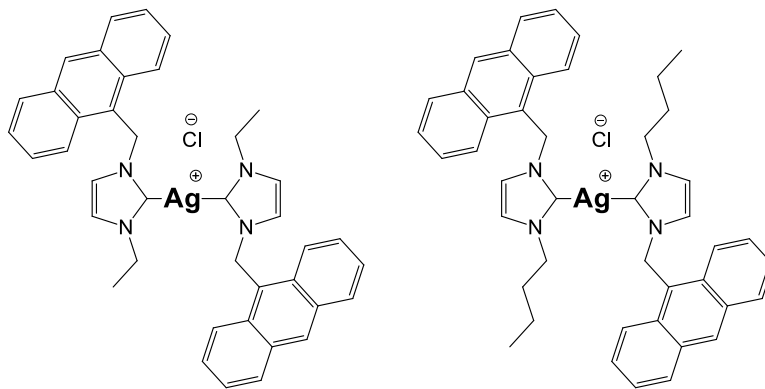


Figure 1. Molecular structure of the studied Ag(I)-bisNHC complexes.

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**DETERMINATION OF ANIONS IN POSTMORTEM SAMPLES FOR FORENSIC EVALUATION**

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Ion chromatography (IC) is an analytical technique for the determination of inorganic and organic anions and cations. In the present study, a new method with IC was developed for separation and quantification of ten anions (fluoride, chloride, bromate, chlorite, nitrite, bromide, chlorate, nitrate, phosphate and sulfate) in postmortem samples, in particular drowning water, pleuric liquid and gastroduodenal content.

The analytical method is simple, fast, accurate and selective for the separation and quantification of ten anions. This first application for forensic purposes showed interesting preliminary results. The results obtained allow to observe the presence of ion exchange processes at the lung interface with the surrounding environment. This element seems to be useful for providing a diagnosis of drowning and an estimation of the submersion time and the postmortem interval.

**FROM THE STREETS TO THE LAW-ENFORCEMENT  
AUTHORITIES: A RAPID AND SENSITIVE UHPLC-MS/MS  
METHOD FOR THE DETERMINATION OF 13 ILLICIT  
SUBSTANCES IN DRUG SEIZURES**

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The European Drug Report 2019 showed that the consumption of illicit drugs in Italy is rapidly growing: about 30% of the Italian population (aged 15-64 years) has used illicit drugs at least once in their life, and 10% in the last year [1]. In the Lombardy region, 59% of the anti-drug operations were registered in the province of Milano. In 2018, 2557 people were reported for drug trafficking and 131 for the criminal association. The area of Milano recorded the second peak (after that of 2009) of drugs seized in the last decade, equal to 58% of those seized in the whole regional territory, for a total of kg 3706.5 [2]. This involves a high workload for the analytical laboratories of law enforcement officers, which give pretty long average times for the analysis's reports (an average time of 20 days for persons reported under arrest and 150 days for people reported on the loose). Thus, aiming to reduce the response time of analyses and support the law enforcement officers, in 2021, an agreement between the Department of Earth and Environmental Sciences (University of Milano-Bicocca) and the Prefecture of Milano was signed for the determination of psychotropic and misuse substances. For this purpose, a rapid and sensitive method has been set for the quantification of the most used illicit substances (cocaine, heroin, 6-MAM, morphine, amphetamine, methamphetamine, MDMA, ketamine, GHB, GBL, LSD, trans-D9-THC, and THCA), derived from street seizures by the law enforcement. This procedure comprises the registration phase of seizures through homemade software, the preparation of the samples, and their analysis by a UHPLC-MS/MS method developed and validated at the analytical laboratories of the University of Milano-Bicocca. The last step includes the final drafting of the analysis report sent to the Prefecture within 24 hours for urgent analyses and within three days for routine ones.

Here, we present the activities description and the results of the agreement's first year.

[1] <https://www.emcdda.europa.eu/edr2019>

[2] <https://antidroga.interno.gov.it>



**DETERMINATION OF CANNABINOIDS IN MILK BY A NEW METHOD BASED ON MICRONIR AND CHEMOMETRICS**

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The administration of antibiotics, having the objective of eliminating pathogens contracted by cattle during the lactation period, is the main remedy of dairy farms, despite leading to a potential presence of drug residues in milk that goes to decrease its quality and make it unavailable for marketing. An innovative method of control for various diseases, that doesn't involve antibiotic treatment, could be the use of feed containing hemp. Cannabinoids found in hemp could counteract the occurrence of various infections contracted by cattle, such as mastitis, due to their anti-inflammatory and antioxidant properties. The aim of this work is to highlight the potential of near-infrared (NIR) spectroscopy for in situ monitoring of the main phytocannabinoids ( $\Delta^9$ -THC and CBD), derived from the consumption of hemp containing feed, present in cow's milk, with the help of a chemometric approach. The chemometric study facilitates the qualitative and quantitative aspects of NIR spectroscopy, favoring its technological advancement. In this work, PLS-DA prediction models were constructed for the prediction of the presence/absence of the two analytes in milk samples from different dairy farms and multivariate models for the quantification of the two cannabinoids using the PLS regression algorithm. Given the high NER% and Sp% and the low RMSE values obtained, always below 0.24 ng, the PLS-DA classification models were highly sensitive and specific, also showed excellent predictive ability for both  $\Delta^9$ -THC and CBD identification. The PLS regression models demonstrated good precision and accuracy by ascertaining excellent repeatability and reproducibility of the method and, in addition, excellent limits of detection and quantification for both analytes were achieved for these models. Finally, the validity of these models was confirmed by the analysis of unknown samples, having correctly estimated all the samples analyzed. Given the excellent results obtained, it can be affirmed that the MicroNIR/Chemometric platform is a promising instrument for the identification and estimation of  $\Delta^9$ -THC and CBD content in cow's milk. This work is the first step in a larger project, to assess the presence and amount of various cannabinoids present in hemp and their metabolites in raw milk, that is in the primary matrix from cattle.

[1] Esposito, G., De Filippis, D., Maiuri, M. C., De Stefano, D., Carnuccio, R., & Iuvone, T., *Neuroscience letters* 399 (2006) 91-95.

[2] Grasel, F. S., & Ferrão, M. F., *Analytical Methods* 8 (2016) 644-649.

**DEVELOPMENT OF BIODEGRADABLE SAMPLING DEVICES FOR THE ANALYSIS OF ILLICIT DRUG IN ORAL FLUIDS.**

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Psychoactive substances consumption over the years is continually increasing; around 29% of adults in the EU are estimated to have used illicit drugs at least once in their lifetime. In order to assess the active consumption of these substances, it is essential to detect the target analyte (active principle or metabolite) in the biological matrices. Among the different matrices, oral fluid (OF) is an interesting matrix due to its simple and noninvasive sample collection and the good correlation with blood concentration, allowing the detection of recent drug intake [1]. The commercial collection devices for OF, which are typically made of plastic materials, may give incomplete desorption of lipophilic drugs or metabolites thus affecting the real concentration of the target analytes in OF. Furthermore, they are generally destroyed through open burning and incineration, resulting in the emission of dioxins, furans and particulate matter. In the last years, billions of tampons were used and measures to prevent this growing waste are desirable [2,3].

For these reasons, the goal of this work was the development of a new biodegradable sampling device. To date, new soluble and biodegradable sampling devices made with polylactic acid (PLA) and printed with 3D technology have been developed for forensic toxicology applications. PLA can be safely introduced in the mouth, as verified with IR spectrophotometry tests, and easily dissolved in acetonitrile at 50°C. The new device was tested for the simultaneous analysis of natural and synthetic illegal drugs. After the dissolution of the sampling device a clean-up is necessary, so a dispersive liquid-liquid microextraction was performed in order to clean-up and concentrate the sample before the LC-MS/MS analysis. Same procedure was applied to existing collection devices, i.e. Quantisal and Cliniswab, in order to compare the efficiency of the new developed device. Preliminary results are promising, so once optimized the protocol will be validated according to national and international guidelines.

[1] A.L. D’Orazio, et al., *J. Anal. Toxicol.* 45 (2021) 529–536.

[2] T. Gug, et al. *Trends Anal. Chem.* 113 (2019) 301–316.

[3] S. Chojnowska, et al. *Adv. Med. Sci.* 63 (2018) 185–191.

**SUSPECT SCREENING OF NOVEL PSYCHOACTIVE SUBSTANCES USING LC-MS/MS WITH IDA ACQUISITION**

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Novel psychoactive substances (NPS) are a group of substances, mainly of synthetic origin, characterised by toxicological properties extremely dangerous for the users, often unaware about the real threat related with these substances.

The aim of this study was the creation of a robust and versatile *suspect screening method* for traditional drugs and NPS, starting from a target method encompassing 97 analytes. The target compounds belong to different classes including natural and synthetic cannabinoids, cathinones, alkaloids, phenethylamines, arylcyclohexylamines, opioids and tryptamine. For this purpose, an UHPLC system coupled to a hybrid triple quad-linear ion trap MS/MS mass spectrometer was used. Target MS detection was carried out by advanced scheduled MRM (AsMRM) algorithm; a suspect screening method was then created by adding new MRM transitions of other NPS, not included in the target list. The molecular ions and fragments for Q1 and Q3 settings were obtained from HighResNPS [1] and the retention time was predicted by using a QSRR specifically developed. The prediction model used was a MLR between molecular descriptor and rT, they are then normalised, correlated and the best used in the calculation of the prediction equation. IDA acquisition mode that coupled an AsMRM as survey scan and an EPI as dependent acquisition was used. With this strategy, screening capabilities were improved and putative identification of different drugs by library matching may be possible even if standards are not available. Calculated LODs ranged between 0.007 ng/mL for 25C-NBOMe and 0.4 ng/mL for morphine. Oral fluid was considered for testing the method in a biological matrix and excellent results in terms of accuracy, precision and matrix effect were obtained. As a proof of concept, the performance of the *suspect method* was evaluated by analysing a mixture containing 21 reference standards not included in the initial dataset; 19 out of 21 analytes were correctly detected. Rt were generally in accordance with experimental Rt, with an average shift of about 13%.

Finally, the *suspect screening method* was applied to four seizures. MXPR and 3-MMC were identified in these samples, showing that this approach is suitable to detect NPS even in real samples.

[1] <https://highresnps.forensic.ku.dk/>.

**LIQUID PHASE MICROEXTRACTION FOR THE DETERMINATION OF 60 ILLICIT DRUGS IN ORAL FLUID BY HPLC-MS/MS ANALYSIS**

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The use of psychoactive substances is increasing worldwide and remains an important social concern. Even if instruments with high level of performances are available, the development of suitable methodologies to isolate the analytes are of interest [1]. The aim of this work was the development and validation of a liquid phase microextraction method for the determination of 60 illicit drugs belonging to different pharmacological classes in oral fluid (OF). The workflow of the present study consisted of four steps: sample collection through passive drool, centrifugation, parallel artificial liquid membrane extraction (PALME) clean-up and liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) analysis for the identification and quantification of the drugs. PALME is a liquid phase microextraction based on the use of a liquid support membrane which was first described and used in 2013 by Gjelstad et al. [2]. This technique uses a pH gradient to facilitate the mass transfer of uncharged analytes across a liquid membrane with organic support and this allows to extract, from an aqueous biological sample, acidic, basic or neutral molecules [3].

In this work, all the parameters that influence the extraction (extraction time, extraction pH, the composition of the extracting solvent and acceptor solution, the effect of adding salt) were evaluated; the method proved to be suitable for analytes of different classes using small quantities of organic solvent and biological sample and it is an example of green chemistry. The method was subsequently validated following the guidelines of Scientific Working Group for Forensic Toxicology by evaluating parameters such as precision and accuracy, LOD and LOQ, linear dynamic range, matrix effect, recoveries.

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**EXPLOITING A PORTABLE CAPILLARY LIQUID CHROMATOGRAPHY INSTRUMENT FOR *IN-SITU* ANALYSIS OF NATURAL CANNABINOIDS**

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The present research aims to the development of miniaturized capillary liquid chromatography (cap-LC) methods for the separation of natural cannabinoids in cannabis inflorescences. The miniaturization, *viz.* the use of very low quantities of mobile phase, enabled the development of a compact mobile system usable for *in situ* analysis, also according to a green and cost-saving approach. UV detection was used, so that the retention data was the main identification parameter [1]. In particular, a Linear Retention Index (LRI) library was built, allowing for a fast, automatic and reliable identification.

Two setups were explored to achieve the efficient separation of twenty-four cannabinoids: a single column setup which exploited a sub-2  $\mu\text{m}$  packing to increase the chromatographic resolution, and a dual-column setup based on the serial connection of two different stationary phases, each coupled to an UV detector. The latter allowed the determination of two LRI values for each analyte, thus increasing the identification power. Moreover, since two different wavelengths, the ratio of the absorbances measured on each chromatographic trace represented a third identification criterion, thus fulfilling the recommendations of the Scientific Working Group for The Analysis of Seized Drugs (SWDRUG) about the categories of analytical techniques to be used and the minimum number of parameters required for the unambiguous identification of drugs [2]. The obtained results could be used for *in situ* forensic investigations and the determination of different hemp breeding programs, as well as the discrimination of cannabis varieties, also minimizing the consumption of both sample and solvent.

[1] R. La Tella, F. Rigano, P. Guarnaccia, P. Dugo, L. Mondello, *Anal. Bioanal. Chem.* (2022), *In press*.

[2] Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG). Recommendations Edition 8.0.

**CAPPARIS SPINOSA L.: AN IMPORTANT MEDICINAL FOOD PLANT**

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*Capparis spinosa* L. also known as Caper is of great significance as a traditional medicinal food plant. The present work was targeted on the determination of chemical composition, pharmacological properties, and *in-vitro* toxicity of methanol and dichloromethane (DCM) extracts of different parts of *C. spinosa*. Chemical composition was established by determining total bioactive contents and via UHPLC-MS secondary metabolites profiling. For determination of biological activities, antioxidant capacity was determined through DPPH, ABTS, CUPRAC, FRAP, phosphomolybdenum, and metal chelating assays while enzyme inhibition against cholinesterase, tyrosinase,  $\alpha$ -amylase and  $\alpha$ -glucosidase were also tested. All the extracts were also tested for toxicity against two breast cell lines. The methanolic extracts were found to contain highest total phenolic and flavonoids which is correlated with their significant radical scavenging, cholinesterase, tyrosinase and glucosidase inhibition potential. Whereas DCM extracts showed significant activity for reducing power, phosphomolybdenum, metal chelation, tyrosinase, and  $\alpha$ -amylase inhibition activities. The secondary metabolites profiling of both methanolic extracts exposed the presence of 21 different secondary metabolites belonging to glucosinolate, alkaloid, flavonoid, phenol, triterpene, and alkaloid derivatives. The present results tend to validate folklore uses of *C. spinosa* and indicate this plant to be used as a potent source of designing novel bioactive compounds.

1. H. Saleem, U. Khurshid, M. Sarfraz, I. Ahmad, A. Alamri, S. Anwar, A.S. Alamri, M. Locatelli, A. Tartaglia, M.F. Mahomoodally, S.A. Zainal Abidin, N. Ahemad, Food and Chemical Toxicology 155 (2021) 1-7, article 112404

**SIMULTANEOUS DETERMINATION OF POLYPHENOLIC COMPOUNDS IN GRAPES SUPPLEMENTS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY–PHOTO DIODE ARRAY DETECTOR**

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Grape byproducts are a rich source of phenolics having immense medicinal properties, but usually wasted from juice/wine processing industries. The present study aims to identify and quantify the major phenolic compounds of extracts prepared from grape cultivars supplements. The major phenolic compounds were characterized in both ethanolic and aqueous extracts, optimizing the extraction time and temperature. The obtained final extracts were then analyzed by High Performance Liquid Chromatography coupled with photodiode array detector (HPLC-PDA). HPLC analyses were performed on a Waters liquid chromatograph equipped with a model 600 solvent pump and a 2996 photodiode array detector (PDA). A C18 reversed-phase packing column (Prodigy ODS(3), 4.6 × 150 mm, 5 μm) was used, and the column oven was set at 30 ± 1°C. The UV/Vis acquisition wavelength was set in the range of 200–500 nm. The quantitative analyses were achieved at maximum wavelength for each compound. The injection volume was 20 μL. The separation was performed using water and acetonitrile (both acidified with CH<sub>3</sub>COOH) in gradient elution mode. The mobile phases were directly *on-line* degassed. The optimization of the extraction and later the analysis in HPLC, allowed the quantification of different compounds within the commercially available grape seed supplements. Overall, supplements are rich sources of phenols and are a source of antioxidants. Future studies could be carried out to evaluate the *in vivo* bioavailability of the phenolic compounds present in the extracts.

**EXPOSURE ASSESSMENT OF NITRITE AND NITRATE INTAKE FROM LEAFY VEGETABLES CONSUMPTION THROUGH MARGIN OF SAFETY (MoS) EVALUATION**

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Nitrite and nitrate levels in leafy vegetables are a matter of concern since nitrate accumulates so much in these foods and some authors also reported high nitrite levels. In this study, 274 samples of leafy vegetables samples (spinach, lettuce, chard and wild rocket) were analysed using ion chromatography for determining nitrite and nitrate. Low and high exposure scenario were considered for each product type using the reference data available from the INRAN-SCAI 2005-06 Italian National surveys on food consumption. Nitrite and nitrate are not genotoxic/carcinogenic, so, the Margin of Safety (MoS) was evaluated, using 1 as the minimum requirement and 100 as a protective threshold. The MoS was calculated as the Admissible Daily Intake (ADI)/Estimated Exposure Dose ratio, for toddlers and adults, considering 12 and 70 kg as reference b.w., respectively, as established by EFSA. Regarding nitrite, 4 MoS values were lower than 1 under high exposure scenario. All these values were related to toddlers population and were obtained for fresh-cut spinach and chard (0.8 and 0.9, respectively), frozen spinach (0.4) and lettuce (0.7). Regarding the protective MoS value of 100, only 8 values out of 38 (21%) resulted higher. Obviously, the general framework is more reassuring for adults unless a very low value, worthy of attention, was obtained for frozen spinach consumption under high exposure scenario (2.6). Regarding nitrate, only 1 MoS value was lower than 1 (fresh lettuce, toddlers, high exposure: 0.5). Moreover, it is worth mentioning that 2 MoS values calculated for toddlers under high exposure scenario were close to 1: fresh-cut spinach and chard (1.6 and 1.1, respectively). Regarding the protective MoS value of 100, 9 values out of 40 (22.5%) resulted higher. The results suggest both the introduction of legal limits for nitrate in Swiss chard and nitrite in leafy vegetables, and the intensification of official controls, especially regarding the levels of nitrate in wild rocket (11 samples out of 44 analysed with nitrate concentration higher than the EU limit). Another significant remark is the greater mean concentration of both nitrite and nitrate in fresh-cut samples. This finding suggests the development of further research aimed at identifying and evaluating the causes of such increases in these products.

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**OXYSTEROLS FINGERPRINTING BY MEANS LC-MS/MS: A TOXICOLOGICAL MARKERS IN ZEBRAFISH EMBRYOS**

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Zebrafish is an *in vivo* model used in toxicology to estimate the effects of xenobiotics and their teratogenic consequences; this animal model presents several advantageous features as high fecundity, rapid embryonic development (24 h) and external fertilization [1]. The knowledge of the oxysterols profile in zebrafish, during early embryonic stages, provides important information on the role and biological function of these molecules. The lipidomic analyses in zebrafish early-life stages could represent a reliable tool for studying the global lipid profile during embryos development; however, the knowledge about the sterols derivatives during the embryogenesis of these vertebrates remains limited [2]. An investigation focused on the profile of the oxysterols, during the early embryonic stages of the zebrafish, could provide information on the amount, roles, and biological functions of these molecules.

The aim of this work was the development of a LC-MS/MS method for the study of oxysterol fingerprint in order to understand the metabolic pathway modification during embryos growth [3]. Moreover, the exposure to propylparaben (PP) and triclocarban (TCC) was also investigated, showing some interesting differences in treated zebrafish embryos for 24-OH and 27-OH. These analytes followed a different distribution, with an increase in TCC treated embryos and a reduction in zebrafish embryos exposed to PP at 24 h post-fertilization (hpf). The results prompt the hypothesis that EDCs can modulate the oxysterol profile in the zebrafish model and that these variations could be potentially involved in the toxicity mechanism of these emerging contaminants.

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**PHARMACEUTICAL CONTROL AND BATCH RELEASE OF DERIVED BLOOD PRODUCTS BY MICRONIR/CHEMOMETRICS**

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In this study, a practical, timely and easy method for the quality control of blood derived products is proposed. It is now known to all that MicroNIR spectrophotometers permits to analyze several matrices without any samples pre-treatment ensuring rapid and intelligible results due to the coupling with chemometrics. [1,2]

Blood products include a wide range of pharmaceuticals that are obtained from voluntary donors of blood or plasma, through industrial manufacturing processes. Like other pharmaceuticals, biological medicines must ensure high quality and efficacy with the minimal risk for potential recipients. Because of their complexity and variability, several analytical tests must be performed for their regulation and batch release. However, the actual analytical protocol requires high expertise to handled samples and guarantee the absence of potential contaminations. In addition, the identification of novel manufacturing strategies is a constant challenge for the National Authority involved in pharmaceuticals control.

In this study, the ability of an easy-to-use spectrophotometer controlled through a low-power wireless (Bluetooth) interface, the MicroNIR, was tested to investigate biological medicines. Spectra were collected directly on the sealed flasks to avoid any potential biological samples contamination. The confirmed analyses were carried out in the laboratories of National Center for Drug Control and Evaluation (CNCF) for the pharmaceutical control and batch release. All MicroNIR data were processed by chemometrics, and a model of prediction was finally developed. Results demonstrate that the Micronir is able to discriminate albumin according to the concentration and as a function of the different provider, suggesting the possibility to evaluate differences in the residuals due to the manufacturing process. The investigation of unknow samples processed by the novel Micronir platform, confirmed that the developed prediction model may be considered as an effective and promising tool in the quality assurance of biological pharmaceuticals. In fact, it is able to evaluate stability, efficacy and safety of pharmaceuticals that reach the patients.

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**MICRONIR/CHEMOMETRICS: A NEW ANALYTICAL PLATFORM FOR CANNABINOIDS ANALYSIS**

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Cannabis sativa L. (hemp) plants have been used all over the world since ancient times due to their multifunctional characteristics, displaying many important biological/pharmacological activities in humans.<sup>1</sup>

This work, aims to implement a new analytical platform based on MicroNIR spectroscopy combined with chemometrics for the quantification of delta-9-tetrahydrocannabinol ( $\Delta$ 9-THC) and cannabidiol (CBD) in different hemp plants, in order to accomplish specific regulatory legislation according to the country.<sup>2</sup>

Spectroscopic techniques proved to be promising in characterizing complex matrices and offered the advantages to be easy-to-use and non-destructive without requiring any pretreatment. In addition, the coupling with chemometric tools will allow for multivariate statistical analysis which provides models of prediction for qualitative and quantitative response with the required accuracy.

The innovation of this approach consists in an ultra-compact and portable device operating in the Near Infrared region, totally controlled via Bluetooth to a laptop. The peculiarity of the portable NIR analytical approach is to bring the laboratory to the "field" obtaining results quickly and comparable to those obtained in the laboratory, since the acquisition time and the instrumental response require only few seconds.

The platform is entirely developed with the aim of simplifying the evaluation of the content of  $\Delta$ 9-THC and CBD in the plants and to facilitate the rapid interpretation of results. All the experiments were carried out to develop a model of prediction as robust as possible. Principal Component Analysis (PCA) and Partial Least Square (PLSr) allowed to discriminate hemp plants according to the presence of CBD and THC. The latter will have to be sensitive and accurate to be used as a first level test in forensic science, and allow to screen suspicious samples for confirmatory analysis using reference procedures.

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**FAST LC-MS/MS METHOD FOR THE DETERMINATION OF PHYTOCANNABINOIDS IN OILY BASED PREPARATIONS**

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The use of cannabis as a medicine has not been rigorously tested through clinical studies mainly due to the production and government restriction related to its use.

Oily-based products are obtained through the production of oleolites with different cannabis cultivars, for this reason a rapid procedures that allow an accurate (precise and true) quali-quantitative determination of the main active ingredients were requested.

We validated a method for the simultaneous determination of tetrahydrocannabinol (THC), cannabidiol (CBD), cannabinol (CBN), cannabigerol (CBG), tetrahydrocannabinolic acid (THCA), cannabidiolic acid (CBDA), and tetrahydrocannabivarin (THCV) in real oily-based pharmaceutical formulation (more of 70 different formulation) in order to verify the accuracy of the cannabis preparation.

The validated method shows a good linearity within the range 0.25–1000 ng/mL, while precision and trueness (intra- and inter-day) were below <13.25% and 7.59%, respectively.

The accuracy, sensitivity and the absence of matrix effects allowed the use for routine analyses and the quantification of seven phytocannabinoids in less than 10 min with an overall HPLC run of 15 min.

The proposed method shows a very green profile based on Green Analytical Procedure Index (GAPI).

**DETERMINATION OF PHTHALIC ACID ESTERS IN VEGETABLE OILS BY DIRECT INJECTION AND CRYOGENIC-MODULATION GC×GC QqQ MS**

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Phthalic acid esters (PAEs) are a large group of compounds, commonly present in plastic products, as additives to increase softness, flexibility, and durability, as well as in medical devices, adhesives, paints, printing inks, baby toys, personal care products, and cosmetics. Concerning toxicological aspects, different studies have suggested that PAEs would act as endocrine-disrupting compounds, being able to negatively modulate hormonal functions and pathways, and they have been also correlated to several cancer forms. Nevertheless, there are still no limits for these contaminants in food, although the European Commission Regulation (EU 10/2011) has restricted some of these in food contact material with specific migration limits.

The aim of this study was to develop and validate a simple and direct method for the analysis of 9 targeted PAEs in edible vegetable oils by means of cryogenic-modulation comprehensive two-dimensional gas chromatography coupled with triple quadrupole mass spectrometry (CM GC×GC-QqQ-MS/MS). The method was characterized by the absence of any sample preparation step; the oil was only diluted with limited volumes of hexane (less than 1 mL) before the injection into a programmed temperature vaporization (PTV) injector. Thanks to the cryo-focusing capabilities of the modulator, and the QqQ-MS/MS (in the multiple-reaction-monitoring mode), the method has high sensitivity and specificity, and allowed the contaminants determination with no need for analyte pre-concentration.

Finally, if compared to other existing methods, this methodology greatly reduces solvent consumption and waste materials production. The developed method was characterized by good repeatability values (lower than 10%), limit of quantification values ranging between 0.06 and 2.10 mg kg<sup>-1</sup>, and accuracy values ranging from -9.2 and 10.4 %

**SUPERCRITICAL FLUID CHROMATOGRAPHY COUPLED WITH TANDEM MASS SPECTROMETRY METHOD FOR THE ANALYSIS OF OXYGEN HETEROCYCLIC COMPOUND IN FRAGRANCES**

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Oxygen heterocyclic compounds (OHCs) are secondary metabolites mainly present in the non-volatile fraction of cold-pressed *Citrus* essential oils. Under this denomination coumarins, furocoumarins and polymethoxyflavones are included. These compounds possess numerous beneficial properties for human health; however, the interaction of furocoumarins with UVA rays could be toxic to human health. Due to their photoactivity, furocoumarins levels are constantly monitored by opinions and regulations issued by the International Fragrance Association. According to European Commission, a limit of 1 ppm for each furocoumarins in finished leave-on product could be considered safe for human health.

The aim of this research was the development of an environmentally rapid, and sensitive friendly method using the supercritical fluid chromatography-triple quadrupole mass spectrometry (SFC-QqQ/MS) technique, for the determination of OHCs in fragrances. For this purpose, seven chromatographic columns packed with different stationary phases were tested. Between those, F5 column allowed the best baseline separation of OHCs in eight minutes with the use of less than 10% of methanol.

Calibration curves of twenty-eight OHCs standards were constructed. Linearity, limit of detection (LoD), limit of quantification (LoQ), accuracy, intraday and inter-day precision were then calculated. All the validation parameters resulted satisfactory, with low LoQs (0.0014 and 0.1536 mg L<sup>-1</sup>) and LoDs (0.0004 and 0.0470 mg L<sup>-1</sup>), that could allowed the quantification of OHCs even when they are contained at trace level in fragrances suggested by European Commission.

The new developed method permits the reduction of solvent consumption and overall analysis time, while keeping a high separation power as well as good identification and quantification capabilities through the use of tandem MS data of the OHCs in the fragrances.

## COMPARISON OF LIPASE IMMOBILIZATION METHODS ON SPENT COFFEE GROUNDS (SCG) SUPPORTS

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Enzymes are currently used in a wide variety of industries and some of the most exploited are the lipases thanks to their unique versatility [1]. In nature they principally hydrolyze triglycerides, but they are also able to catalyze in vitro esterification, acidolysis, interesterification, transesterification, and aminolysis, together with a collection of the so-called promiscuous reactions [2]. However, how every other enzyme, lipases have a low stability and immobilization procedures are often applied to enhance their performances. The most common immobilization methodologies are covalent immobilization, physical adsorption, cross-linking, encapsulation, and entrapment on both synthetic and natural derived materials [3]. Although synthetic materials have many advantages, they are difficult to obtain and require higher additional costs. On the other hand, material derived from natural sources, like spent coffee grounds (SCG), have favorable physical-chemical characteristics and are also largely available and inexpensive. Coffee, in particular, had an estimated production of over 10 billion kg per year in 2020 [4] and is responsible for generating large quantities of residues that may represent a great pollution hazard if discharged into the environment. Therefore, the aim of this study is focused on the ideation of a suitable carrier for *Candida Rugosa Lipase* (CRL) from SCG. With this purpose SCG was pretreated in different ways to assure the best characteristics for each methodology of immobilization. The materials were characterized by SEM, IR, and elemental analysis. Subsequently four different kinds of immobilization methods were evaluated: (i) adsorption, (ii) crosslinking, (iii) covalent binding after periodate oxidation of the support and (vi) covalent binding after activation of the support with glutaraldehyde (GLU). The optimal immobilization conditions were determined on the base of yield, efficiency, and recovery.

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**CAPILLARY ION CHROMATOGRAPHY AS “GREEN”  
TECHNIQUE FOR THE DETERMINATION OF NITRITE AND  
NITRATE IN LEAFY VEGETABLES**

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Nitrate and nitrite are two compounds present in the environment since they take part in the nitrogen cycle. In agriculture, nitrate is widely used as inorganic fertilizers. Due to this high anthropic impact and fossil fuel combustion, during the second half of the 20th century, nitrate accumulated in the environment drastically. The toxic effects due to high intake of nitrite/nitrate, such as the possible formation of N-Nitrosamines, and methemoglobinemia, are well-known. Leafy vegetables are characterized by high nitrate concentration, so that a great part of nitrate daily intake derives from vegetable consumption. Regarding nitrite, it can be present in vegetables only at low concentration, deriving from endogenous nitrate reduction. However, recent articles reported about the possible presence of high levels of nitrite in leafy vegetables (up to 197.5 mg kg<sup>-1</sup> in spinach).

Within official controls, ion chromatography with suppressed conductivity detection is the most used and well-established technique for the analytical determination of nitrite and nitrate. In this study, we developed and validated an alternative method by capillary ion chromatography (CIC) coupled to conductivity detection, for this type of analytical determination, in the view of “Green Chemistry”. Indeed, capillary ion chromatography is characterized by very low reagent consumption. The method was fully validated following the most updated guidelines and according to the European Normative, for the analysis of different types of leafy vegetables, such as spinach, lettuce, chard and wild rocket. The method is characterised by high sensitivity (LOQs: 3.65 and 2.07 mg kg<sup>-1</sup> for nitrite and nitrate, respectively), selectivity towards interferences higher than the traditional ion chromatography method, recovery percentages in the range 98.8% - 100.8%, excellent repeatability (RSD% < 3.4%, n=18), low measurement uncertainty (2.4% and 2.6% for nitrite and nitrate, respectively), and large application field, since other than leafy vegetables, it is also applicable for meat, seafood, dairy products, animal feeds and other vegetables analysis. The proposed method may be considered as a valid tool of investigation for organisms in charge of food inspections, in the perspective of “Green Chemistry” [1].

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**RECYCLING OF CIGARETTE FILTERS: RECOVERY OF CELLULOSE ACETATE FOR THE PREPARATION OF LOW-COST SORBENTS**

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One of the *Green Chemistry* goals is the development and application of new sorbent materials having the characteristics of eco-compatibility, low toxicity, recyclability, and biodegradability. Several bio-based polymers, such as cellulose and cellulose acetate, are obtained from biomass or recycled waste. From this perspective, filters recovered from cigarette butts can be an important source of these two biopolymers, considering the fact that, worldwide, around 6 trillion cigarettes are smoked every year, for a total waste from 340 to 680 million kilograms [1].

With such a goal in mind, this study has been aimed at: *i*) developing an effective procedure to recover cellulose acetate from filters of cigarette butts; *ii*) employing the recycled biopolymer to prepare a new sorbent to be used for solid-phase extraction (SPE) on analytical scale, and for the water treatment at industrial level. The first step of the work was focused on obtaining clean filters from cigarette butts. To this end, after eliminating wrapping paper and unburned tobacco, filters were submitted to a cleaning process. Most of impurities, produced during the burning of cigarettes (more than 7000), were washed with hot water and hot ethanol (non-solvents); then, filters were dissolved in acetone (good solvent) containing activated carbon for the complete removal of undesired residues. The cleaning effectiveness was verified by analyzing the polymer solution with UV spectroscopy (190-800 nm) and comparing the spectral profiles with those obtained from solutions of pristine and dirty material. After solvent evaporation, cellulose acetate was recovered and analyzed by ART spectroscopy (400-4000 cm<sup>-1</sup>) and thermal analysis, showing how its physicochemical properties reflect those of the pristine material. The cellulose acetate so recovered was used to realize a composite material with activated carbon using the phase inversion technique. The sorbent was then characterized and applied for water remediation purposes, showing good adsorption capabilities towards organic contaminants.

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## PREPARATION AND APPLICATION OF CYCLODEXTRIN-BASED NANOSPONGES AS SUSTAINABLE SORBENTS

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Green Analytical Chemistry (GAC) [1] encourages the use of direct analysis. As a matter of fact, sample pre-treatment is the weakest link in the analytical chain, taking up most of the time and involving the most polluting steps of the entire procedure. However, for the trace or ultra-trace determination of analytes in complex matrices it is often the only choice.

Solid-phase extraction (SPE) is one of the most used techniques for the treatment of liquid samples. Lately, the research in this field has focused on the development of sorbent materials and procedures that meet the GAC criteria. Cyclodextrins (CDs) represent an excellent starting point for synthesizing natural sorbents since they are obtained from starch, one of the most available and cheapest biomasses after cellulose. CDs are cyclic oligosaccharides made up of D-glucofuranose units whose 3D structure is characterized by a hydrophobic internal cavity suitable to form host-guest complexes with target analytes [2]. The numerous hydroxyl functionalities present on the external surface allow CDs to act as polyfunctional monomers to form nanosponges (NS) [2], insoluble nanostructured polymers that, in this work, were obtained through a green synthesis based on the use of acid citric as the crosslinker.

Once synthesized, the cyclodextrin nanosponges (CDNSs) were characterized (SEM and thermal analysis) and applied to extract 13 chiral and achiral pesticides from environmental water samples by means of dispersive-SPE. All extracts were analysed by HPLC-MS/MS, on both C18 and polysaccharide columns to evaluate the CDNS's potential in chiral discriminations.

The possibility of using these materials in SPE procedures is particularly interesting due to their properties and potentialities that are still unexplored.

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## A CHITOSAN-BASED, EDIBLE BIOFILM FOR FOOD WRAPPING: FROM FORMULATION TO FUNCTIONALITY

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An edible biofilm for food wrapping, was developed through an eco-friendly and low-cost process. The formulation was optimized to achieve the functionality of protecting food and prolonging its shelf-life, with an alternative biomaterial to conventional plastics<sup>1</sup>. The biofilm was obtained by deposition and drying on slab of a layer (300 µm) of a blend with film-forming and antimicrobial properties. The key constituents of the blend are chitosan, reclaimed from fish waste, and a mix of essential oils from aromatic herbs (i.e. *Thymus vulgaris* L.) as well as natural additives (including glycerol, nopal mucilage and hydrolyzed silk proteins) exerting the function of plasticizers, emulsifiers and waterproofing. Chitosan (food-grade with DD=95%) was extracted from crustacean waste, preliminarily dried and powdered. The chitin contained in the powder was demineralised with HCl, deproteinized with NaOH and deacetylated with 40% NaOH solution. The implementation of microwave and sonication in each step maximized the yield (20%), reducing the amount of conventionally used chemicals, as well as times and temperatures of the process. The testing of biofilm by standard methods showed interesting barrier, mechanical and bacteriostatic properties. In particular, the moisture content (MC) was 9.11±0.09% and the water vapour permeability (WVP) was 11.2×10<sup>-11</sup> g m<sup>-1</sup> s<sup>-1</sup> Pa<sup>-1</sup>. The elongation at break (EB%) resulted 31.3±1.26%. The biofilm is effectiveness in prolonging the shelf-life of different hard cheeses. The microbiological quality of control (C: without biofilm) and treated (T: with biofilm) samples, during storage at 22 °C, was assessed. After twenty days the mesophilic bacteria count was 1,42±0.09×10<sup>6</sup> CFU/g (T samples) and 2,64±1.12×10<sup>6</sup> CFU/g (C samples) and the acidophilic microorganisms count was 0,47±0.05×10<sup>6</sup> CFU/g and 2,42±0.09×10<sup>6</sup> CFU/g (T vs C samples). The biofilm developed resulted high-performing in food preservation with the added value deriving from its raw material coming from fish industry waste.

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**POTENTIAL OF RSLDE: A GREEN TECHNIQUE FOR THE EXTRACTION OF BIOACTIVE MOLECULES FROM PLANT BIOMASS AND INDUSTRIAL PROCESSING WASTE**

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This study highlights the potential of the Naviglio extractor (NE), an innovative solid-liquid extraction technique, better known in the literature as RSLDE (Rapid Solid-Liquid Dynamic Extraction). Solid-liquid extraction is based on a “trial and error” process, in fact, there are currently no universal models of phenomenology that can anticipate the results and therefore the best a posteriori extraction conditions must be studied for each individual application. In fact, only after completing the extraction process is it possible to scientifically classify the results, which can only be reproduced while maintaining all the operating conditions (matrix to be extracted, granulometry, humidity of the material, extracting solvent, temperature and so on). In conventional techniques, such as maceration and percolation, solid-liquid extraction occurs essentially for two effects: diffusion and osmosis and for this reason it can be defined as passive, as it is necessary to wait for the slow diffusion times to complete the process. The increase in temperature and the occasional agitation of the extraction system are the only parameters that can speed up the process. Unfortunately, if on the one hand the increase in temperature helps to speed up diffusion (Fick's Law), on the other hand it degrades the molecules extracted from the plant matrices, which in most cases are thermolabile. On the contrary, RSLDE carries out an innovative solid-liquid extraction process, as it makes the phenomenon active, that is, with the pressure-depression effect, it forces the release of substances that are not chemically linked to the solid matrix and accelerates its extraction. Therefore, RSLDE can bring about a number of improvements in the field of solid-liquid extraction, such as total recovery of alcohol, the re-extraction of the exhausted matrix using an extraction with increasing polarity, more exhaustive extractions than the conventional techniques. Furthermore, RSLDE can be performed both on plant matrices and on waste material showing a greater efficiency and a lower environmental impact of this technique.

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**EXTRACTION EFFICIENCY COMPARISON BETWEEN DEEP EUTECTIC AND ORGANIC SOLVENTS IN A MATRIX SOLID PHASE DISPERSION METHOD FOR THE ANALYSIS OF TRIAZOLIC FUNGICIDES IN TOMATOES**

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Although organic solvents are useful in extracting pesticides from food samples, most of them have inherent drawbacks like high toxicity, non-biodegradability and high cost. Using green solvents, such as deep eutectic solvents (DESs), is a viable strategy to reduce sample preparation environmental secondary pollution [1]. Matrix solid-phase dispersion (MSPD) is a versatile extraction technique for pesticide food residues analysis. Using DESs as extractive media moves MSPD closer to the green analytical chemistry, but their negligible vapor pressure could negatively affect method sensitivity [2].

In this work, a DES-based MSPD extraction was performed to detect five triazolic fungicides in tomato samples. In order to improve method sensitivity, a liquid-liquid extraction (LLE) was carried out from the DES extracts using a minimum amount of organic solvent.

Method optimization involved DES and organic solvent nature, organic solvent-to-DES ratio (v/v), nature of dispersant, sample-to-dispersant ratio (w/w) and extraction solvent volume.

Following the optimized method, 0.2 g of sample were crushed with alumina in a 1:5 matrix-to-dispersant ratio (w/w). Analytes were eluted through a cartridge using 4.5mL of choline chloride-ethylene glycol (1:2 molar ratio) based DES. Then, 2 mL of ethyl acetate were used to recover fungicides from DES extract in a LLE procedure, before HPLC-MS analysis.

Intra-day and inter-day precision, limit of detection, limit of quantification, accuracy and linearity range were considered for method validation.

Finally, the extraction method efficiency was compared using DES and some common organic solvents as extraction media.

The developed method reduced the use of organic solvents in MSPD and the use of DES instead of tested organic solvents provided higher pesticide recovery.

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**FLOW-MODULATED COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY COMBINED WITH TIME-OF-FLIGHT MASS SPECTROMETRY: USE OF HYDROGEN AS A MORE SUSTAINABLE ALTERNATIVE TO HELIUM**

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The present research is focused on the use and evaluation of hydrogen, as a more sustainable alternative to helium, within the context of fast flow-modulation comprehensive two-dimensional gas chromatography-time-of-flight mass spectrometry. In such a respect, a comparison was made between the two mobile phases in terms of speed and overall chromatography performance. All experiments were carried out by using the following column set: low polarity with dimensions 10 m × 0.25 mm ID × 0.25 μm d<sub>f</sub> and medium polarity with dimensions 2 m × 0.10 mm ID × 0.10 μm d<sub>f</sub>.

Fundamental gas chromatography parameters (efficiency, and resolution) were measured under different experimental conditions, using the two carrier gases. Specifically, efficiency was measured in both dimensions, using a probe compound under isothermal conditions; after defining the optimum carrier gas conditions, a mixture of 20 pesticides was analysed to measure resolution, again in the first and second dimension, using a temperature program. It was found that a similar chromatography performance can be attained when using hydrogen, albeit with a circa 25% reduction in analysis time. Finally, a comparison was made between mass spectral profiles obtained analysing the pesticides and fatty acid methyl esters using the two mobile phases.

**MOLECULAR TRAPS BASED ON NATURAL ZEOLITES FOR ENVIRONMENTAL REMEDIATION**

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Zeolites are able to adsorb small polar molecules (e.g, acetic acid, formic acid, water, etc)<sup>1</sup>, because of the unbalanced electric field of the extra-framework cations. As a consequence, these crystalline substances can be very conveniently used as molecular traps for the environmental remediation. A further advantage of these materials is that the presence of cations makes them electrically conductive, and therefore the adsorption behaviour can be simply monitored by measuring the intensity of the ionic current moving in the sample<sup>2</sup>. Zeolites may have natural or synthetic origin, however natural zeolites, like clinoptilolite, are widely spread minerals characterized by a very low cost. Here, a sample of natural clinoptilolite has been morphologically (SEM), structurally (XRD) and spectroscopically (FT-IR, EDS) characterized in order to establish its exact composition. Water has been used as a probe-molecule to monitor the gas-phase adsorption process by real-time current intensity measurement.

Clinoptilolite is a natural zeolite type widely available on the market at very low cost. In order to improve the trapping ability of this material, that is based on an intra-pore diffusion mechanism and therefore may have a quite slow kinetics in the case of large grains, the coarse zeolite raw material needs to be homogeneously reduced to a powder with micronic/sub-micronic size<sup>3</sup>.

Here, a coarse clinoptilolite sample, has been first morphologically and structurally characterized by Scanning Electron Microscopy (SEM)<sup>4</sup> and X-Ray Diffraction (XRD), respectively. Then, the raw material has been dry-grinded in order to fully separate the contained lamellar single-crystals stacked in the mineral, thus producing a nano-sized powder. Usually, natural clinoptilolite is a combination of different crystalline solid phases (e.g., zeolites, quartz, etc.), and clinoptilolite is only the main crystalline component of such composite system. Consequently, the exact mineralogical composition needs to be experimentally determined by using X-Ray Diffraction (XRD). According to the literature, the found crystalline phase composition is quite typical for clinoptilolite of natural origin.

The characteristic molar Si/Al value of the sample and the type of contained charge-balancing cations were determined by Energy-Dispersive Spectroscopy (EDS)<sup>5</sup>. According to the EDS spectrum, the specimen contained signals of: K<sup>+</sup>, Ca<sup>2+</sup>, and Mg<sup>2+</sup> (Fe<sup>3+</sup> was present at very low amount). A value of the molar Si/Al ratio close to 5.4 was calculated by using the signals of the two elements (such a value is typical for clinoptilolite).



The clinoptilolite mineral has shown a good capacity for the water adsorption, in particular saturation is reached after ca. one day for an environmental humidity of 75% by weight.

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**UNIHEMP PROJECT: SUPERCRITICAL FLUID EXTRACTION AS GREEN ALTERNATIVE TO TRADITIONAL EXTRACTION OF NATURAL COMPOUNDS**

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The UniHemp project “Use of iNdustrIal HEMP biomass for ENergy and new biocheMicals PProduction” aims to generate a circular economy around the hemp supply chain, in which its waste enters new production cycles, such as that of biochemicals with high added value and renewable energy. The development of advanced and eco-sustainable extraction methods such as Supercritical Fluid Extraction (SFE) fits into this context.

Since the early 1970s, SFE technology has been widely used for a variety of purposes, including the extraction of natural products to obtain low-polarity chemicals [1]. SFE compared to traditional extraction techniques, including maceration, Soxhlet extraction, hydrodistillation, solid / liquid phase, has consistently produced higher yields in less time, low analysis cost and eco-sustainable aspects that demonstrating its advantages over traditional extraction techniques.

CO<sub>2</sub> has the advantage of being non-flammable and non-toxic, and it allows for selective material processing while adjusting the extraction conditions.

In this work, hemp inflorescences (*Cannabis sativa L.*) genotype Carmagnola were extracted using supercritical CO<sub>2</sub> under different pressure conditions.

The maximum oil yield from the inflorescences was obtained by extraction of supercritical carbon dioxide (scCO<sub>2</sub>) at a temperature of 45° C at a pressure of 140 bar.

After developing the SFE setup, the extracts were analyzed by gas chromatography-mass spectrometry (GC-MS).

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**RECOVERY OF PHENOLIC COMPOUNDS FROM STRAWBERRY RESIDUES BY NATURAL DEEP EUTECTIC SOLVENTS**

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The number of wastes produced during the chemical processes and the use of hazardous agents has significantly increased in the years with a drastic negative impact on the environment. However, the ecological sensitivity and the interest in “Green Chemistry” are increasing, thus leading to a decrease in the impact these substances cause on the ecosystem [1]. The replacement of traditional solvents with NADES (Natural Deep Eutectic Solvents) represents an excellent green alternative as they bring numerous advantages such as reusability, biodegradability, non-toxicity, large-scale availability, high thermal stability and ease of preparation; therefore NADES can be considered as eco-compatible solvents [2]. In this work, the performances of traditional organic solvents and NADES were compared for the extraction of phenolic compounds (PCs), with different physicochemical characteristics, from strawberry residues. For this reason, numerous combinations of NADES have been evaluated, optimizing the experimental conditions by experimental design. The samples were then analyzed using HPLC-DAD-MS/MS, which allowed the identification and quantification of numerous PCs in the samples. The obtained results were then analyzed by means of surface response methodologies, showing how green solvents can satisfactorily replace traditional extractions, reducing the impact on the environment.

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**GREEN EXTRACTION OF FLUOROQUINOLONES FROM VEGETABLES FOLLOWED BY HPLC-MS/MS ANALYSIS**

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The intensive use of antibiotics has led to notably widespread contamination not only of water and soil, but also of food chain. Indeed, antibiotic residues in vegetables have been reported because of the use of recycled water for irrigation and the application of sewage sludge, biosolids and manure as soil amendment or fertilizers, and their presence should be monitored in roots/leaves/fruits based on their lipophilic/hydrophilic balance [1].

In this work we present a simple and sensitive method for the determination of both veterinary- and human- use antibiotics belonging to the class of Fluoroquinolones (FQs), namely Marbofloxacin, Levofloxacin, Norfloxacin, Ciprofloxacin, Danofloxacin, Enrofloxacin, and Orbifloxacin from vegetables. These are zwitterionic and hydrophilic compounds ( $\text{Log } P < 2$ ), thus they tend to accumulate in leaves and fruits with high content of water. The herein proposed method involves an ultrasound assisted extraction (UAE) by using an aqueous solution of 20% (w/v)  $\text{Mg}(\text{NO}_3)_2$  with 2% (v/v)  $\text{NH}_3$ . It is well known that  $\text{Mg}(\text{II})$  forms chelates with the deprotonated carboxylic acid group of FQs, thus avoiding consumption of organic solvents. The procedure was developed in freeze-dried spinach, here selected as probe matrix. The influence of different parameters, *viz.* ratio matrix amount/solvent volume, ammonia amount, sonication time and number of cycles, on the extraction efficiency has been studied. The final procedure was performed on 0.5 g (dry weight) and comprised of 3 x 2 min ultrasonic cycles with 6 mL of aqueous solution. To reduce matrix effects resulting from matrix components, the UAE extract was diluted to 50 mL with water (pH 3) and preconcentrated on HLB<sup>TM</sup> cartridges. The elution was performed with 0.1%  $\text{HCOOH-ACN}$  (80:20), directly analysed in HPLC-MS/MS system (MRM mode). Method accuracy was studied by recovery tests at three concentration levels: low (LQC,  $15 \text{ ng g}^{-1}$ ), medium (MQC,  $70 \text{ ng g}^{-1}$ ) and high (HQC,  $150 \text{ ng g}^{-1}$ ), achieving good recoveries in the range 63%-95%, and precision (% RSD) below 20% ( $n=3$ ). The method applicability to other vegetables (e.g., lettuce and cucumber) and its fully validation are ongoing.

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## UHPLC-MS INVESTIGATION ON THE COMPLEXATION AND OXIDATION OF QUERCETIN IN PRESENCE OF SELECTED METAL CATIONS

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Flavonoids are a group of polyphenolic compounds that have received increasing research interest in recent years as display a broad variety of bioactivities, one of the most relevant is their capacity to act as antioxidants. Flavonoids can be employed as convenient sequestering agents [1-3], indeed, metal chelation therapy is often used to treat metal toxicity. In this study, a general approach to the bonding and chelating properties of quercetin (3,3',4',5,7-pentahydroxyflavone, H<sub>5</sub>Que) to metals cations is described. In this framework, the aim of this work was to investigate the complexation of quercetin towards selected metal cations, *i.e.*, Cr(III), Mn(II), Fe(III), Co(II), Ni(II), Cu(II), Zn(II) and Al(III), in aqueous solution, with the aim to verify also the oxidation of the ligand in presence of these metals at 37 °C and at two different pH, *i.e.*, 4 and 7.4. The choice of these metals was related to the knowledge of their speciation profiles, useful to identify the existing complexes. All systems were incubated for 24 hours, and some of these also at different time with the aim to investigate the kinetics of oxidation products formation. At the end of each incubation, the systems were characterized with several instrumental procedures: RP-UHPLC-PDA-ESI-IT-MS (Reversed phase liquid chromatography) to identify and quantify the amount of the products; UV-vis spectrophotometric measurements to confirm the effective formation of metal complexes; ESI-IT-MS (electrospray ionization ion trap mass spectrometry) to verify the species present in the systems; NMR (Nuclear Magnetic Resonance) to identify the chelation sites. Some of the metal cations investigated form with quercetin stable complexes which can prevent oxidation of the ligand, while others catalyze oxidation.

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**A DATA DEPENDENT ACQUISITION-BASED APPROACH FOR THE IDENTIFICATION OF UNKNOWN FAST-ACTING TOXINS AND THEIR METABOLITES IN SHELLFISH**

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Cyclic imines (CI) are marine biotoxins listed among the emerging toxins of European concern characterized by fast-acting toxicity inducing a characteristic rapid death in mouse intraperitoneally injected [1]. From a chemical standpoint, CIs are macrocyclic molecules with imine and spiro-linked ether moieties, and they include spirolides, gymnodimines, and pinnatoxins among others. Their detection in raw and processed shellfish has significantly increased in the last decades in the Mediterranean area, so raising serious concern for seafood safety. In the absence of robust toxicological data, the European Food Safety Authority has required more scientific efforts aimed to assessing their actual distribution in seafood based on validated analytical methods before establishing a maximum permitted level in seafood. As a result, currently, no regulation exists for this group of toxins [2].

In this study, a new untargeted LC-HRMS method based on data dependent acquisition has been conceived and successfully applied in combination with targeted studies for an in-depth investigation of the metabolic profile of shellfish. This approach allowed to discover several novel gymnodimine fatty acid ester metabolites, as well as to implement a new LC-HRMS-based strategy, named “backward analysis”, for discovery and identification of new toxin analogues. The implemented analytical workflow has broadened the structural diversity of gymnodimine family through the inclusion of five new analogues, gymnodimine –F, to -J. Their tentative structural characterization has been also carried out.

In parallel, the study of toxin accumulation in Mediterranean and Galician trophic chain revealed the presence of gymnodimine A in Tunisian shellfish, pinnatoxin G in mussels from the Atlantic coast of Spain (Rías Baixas) and, for the first time, pinnatoxin G in mussels from Sardinia (Tyrrhenian Sea, Italy).

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**APPLICATION OF A NEW INTEGRATED GC-FTIR/MS APPROACH FOR THE UNIVOCAL DISCRIMINATION OF REGIOISOMERS**

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The hyphenation of gas chromatography and mass spectrometry (GC-MS) equipped with an electron ionization (EI) source is the gold standard for identification of unknown volatile compounds in different samples (including food, forensic, flavor and fragrance, petroleum chemistry). The technique affords high sensitivity and selectivity; furthermore, a large number of dedicated databases are available for spectral search. Nevertheless, the use of GC-MS alone has some limitations in geometrical and positional isomers discrimination, because of the high similarity of their mass spectra. Consequently, the results from library search may be not univocal, and more than a single result is often displayed.

In contrast, Fourier Transform Infrared Spectroscopy (FTIR) provides a wealth of molecular information of GC separated analytes, related to the vibration of molecular functional groups, which makes possible the definitive isomer discrimination. Yet the lack of infrared spectral libraries has limited so far a widespread use of GC-FTIR.

In this research, a new GC system coupled to EI MS and to a solid phase FTIR (GC-sd-FTIR/MS) detector was developed, based on post-column flow splitting for parallel MS and IR detection. By means of this integrated instrumentation, it was possible to obtain three complementary information from a single analysis: the linear retention indices (LRI) of the separated compounds, their IR and  $m/z$  ions (fragments). This triple filter approach resulted successful in achieving the confident identification of complex mixture components, by narrowing the spectral search to a restricted elution window. Quality match factors higher than 90% are typically achieved, in an automated and unbiased manner.

**QUANTIFICATION OF BENZOTHAZOLES, BENZOTRIAZOLES AND BENZENESULFONAMIDES IN ENVIRONMENTAL WATERS BY THE SYNERGISTIC COMBINATION OF MEPS AND PTV-GC-MS/MS, OPTIMIZED THROUGH EXPERIMENTAL DESIGN-PCA APPROACHES**

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Benzothiazoles (BTHs), benzotriazoles (BTRs), and benzenesulfonamide (BSAs) are high production volume chemicals that are considered new emerging pollutants because of their health concern and their widespread presence in all the environmental compartments. [1–2]

This study addresses the development of a new method for the quantification of BTs, BTRs, and BSAs in tap water, river water, and wastewater. The presented approach is based on the use of microextraction by packed sorbent (MEPS) combined with programmed temperature vaporization-gas chromatography-triple quadrupole mass spectrometry (PTV-GC-QqQ-MS) operating in tandem mass spectrometry. In this study, the MEPS was used for the first time in the determination of these ubiquitous pollutants.

Combining the use of MEPS with PTV requires a close synergy and the achievement of optimal working conditions necessarily depends on their simultaneous comprehensive optimization. For this reason, all factors of MEPS and PTV have been investigated simultaneously through experimental design, thus offering the analyst response surfaces which, in compliance with the principles of Green Sample Preparation (GSP) [3] allow an informed choice of experimental conditions according to the required analytical performance. Furthermore, in this study we chose to apply principal component analysis (PCA) for this purpose, using score values as a comprehensive response of the experimental results.

The developed method has shown satisfactory figures of merit and offers improvements in terms of eco-compatibility compared to other GC-based protocols already published in the literature. These advances are mainly due to the adoption of MEPS which requires less sample and solvent volumes and allows a reduction of the analysis time leading to an increase in the sample throughput.

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**GAS CHROMATOGRAPHY ANALYSIS OF *CANNABIS SATIVA L.* ESSENTIAL OIL OBTAINED BY USING A NEW MICROWAVE DISTILLATION TECHNIQUE, FOR THE CHARACTERIZATION OF THE VOLATILE FRACTION**

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*Cannabis Sativa L.* is a plant that deserves special attention for its phytochemical and pharmacological properties. The great variety of possible uses attracts interest of many industries, for example in medical and cosmetics fields.

The extraction and isolation of *Cannabis Sativa L.* essential oil was performed on fresh and dried hemp inflorescences. A solvent-free microwave distillation method was optimized: the use of distillate water instead of organic solvents makes the procedure environmentally sustainable. The distilled product was rich in terpenes and terpenoid compounds, responsible of the typical and unique smell of the cannabis plant and involve in anti-inflammatory properties of the cannabis EO. The distillate from different hemp cultivars, including various cultivars, were analyzed by using a gas chromatograph equipped with both mass spectrometer and flame ionization detectors. Peak assignment was established using a reliable approach: reverse match, and linear retention index filter, by using the FFNSC 4.0 mass spectral library. Absolute quantification analytes was performed using an internal standard method applying the FID detector response factors according to each chemical family. An enantio-GC-MS method was also developed in order to evaluate the enantiomeric distribution of ten couples of chiral compounds. [1]

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**CHEMICAL CHARACTERIZATION OF THREE ACCESSIONS OF *BRASSICA JUNCEA L.* BY USING SPME TECHNIQUE**

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Brassica Juncea L. (or Indian mustard) is an oil seed plant used for food and non-food applications, for example in phytoremediation of metal and pesticides. *Brassicaceae* family contains different biological compounds, so it has potential beneficial on human health and it gained a good attention in recent years.

In this work, the SPME extraction was effected on the lyophilized powder of three different accessions of Brassica Juncea L.: ISCI99 (Sample A), ISCI TOP (Sample B) and “Broad-Leaf” (Sample C). Qualitative analysis of the volatile fraction was performed on a gas chromatograph equipped with a single-quadrupole mass spectrometer. Quantitative analysis expressed in terms of percentage area of the compounds was performed by using GC technique coupled to a flame ionization detector (FID). Compounds were identified with the support of the FFNSC 4.0 and the W11N17 spectral libraries, applying both the spectral similarity filter and Linear Retention Index (LRI) filter, a reliable tool for a certain identification in a complex matrices.

The analysis demonstrate an abundant volatile profile, rich in alcohols, aldehydes, esters, fatty acids, ketones, sulfur compounds, and other compound. In addition, non-volatile fraction studies were carried out on this plant: most polyphenols identified were bound to sugars and phenolic acids. These studies suggest insights for future novel applications like supplements and nutraceuticals in food matrices. [1]

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**DEVELOPMENT OF A MULTIPLE REACTION MONITORING TANDEM MASS SPECTROMETRY METHOD TO MONITOR SARS-COV-2 PROTEINS FROM NASOPHARYNGEAL SWABS AND SERUM PROTEINS IN VIRUS AFFECTED SUBJECTS**

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Numerous reverse transcription polymerase chain reaction (RT-PCR) tests have emerged over the past year as the gold standard for detecting millions of cases of SARS-CoV-2 reported daily worldwide and the response to the virus. However, problems with critical shortages of key reagents such as PCR primers and RNA extraction kits and unpredictable test reliability related to high viral replication cycles have triggered the need for alternative methodologies to PCR to detect specific COVID-19 proteins. Several authors have developed methods based on liquid chromatography with tandem mass spectrometry (LC-MS/MS) to confirm the potential of the technique to detect two major proteins, the spike (S) and the nucleoprotein (N) of COVID-19 [1-3].

An S-Trap mini spin column digestion protocol was recently developed for sample preparation prodromal to LC-MS/MS analysis in multiple reactions monitoring (MRM) ion mode to obtain a comprehensive method capable of detecting different viral proteins and some of those proteins involved into the virus response [4]. The developed method was applied to n. 81 oro/nasopharyngeal swabs submitted in parallel to quantitative reverse transcription PCR (RT-qPCR) assays to detect RdRP, the S and N genes specific for COVID-19, and the E gene for all *Sarbecoviruses*, including SARS-CoV-2 (with cycle negativity threshold set to 40). A total of 23 peptides representative of the six specific viral proteins were detected in monitoring 128 transitions found to have good ionic currents extracted in clinical samples that reacted differently to the PCR assay. The best instrumental response came from the FLPFQFGR sequence of spike [558-566] peptide used to test the analytical performance of the method with good sensitivity with a low false-negative rate. A panel of more than 40 proteins

involved in the inflammatory proteins and coagulation factors was monitored in 100 human sera affected by SARS-CoV-2. Transition monitoring using a targeted MS approach has the great potential to detect the fragmentation reactions of any peptide molecularly defined by a specific amino acid sequence. This aspect offers the extensibility of the LC-MRM/MS approach to any viral sequence including derived variants and the monitoring of human serum proteins involved in the response to any pathogen.

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**DEVELOPMENT OF A NEW METHOD FOR THE ENANTIOSEPARATION OF NINE CHIRAL PESTICIDES IN AQUEOUS AND BIOLOGICAL SAMPLES USING LC/DAD AND HPLC/MS/MS**

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Since 2007, 43% of newly commercialized agrochemicals contains chiral molecules and most of them have been launched on the market as racemates or diastereomeric mixtures (eutomer and distomers) [1]. Stereoisomers of agrochemicals not only show different efficiency and toxicity, but they undergo also different environmental transformations, especially in case of microbial degradations [2]. In order to enantioselectively verify the harmful residues in the environment, as well as their effects towards people occupationally and non-occupationally exposed, the development and validation of chiral separation methods is an urgent necessity. The first part of this work was aimed at optimizing a new enantioselective liquid chromatographic method, based on both UV ( $\lambda = 220$  nm) and MS/MS detection. Unlike conventional methods focused on one or two analytes, the one described here was able to separate nine chiral agrochemicals (18 chromatographic peaks) on the Lux cellulose-2 column with cellulose tris(3-chloro-4-methylphenylcarbamate) as a chiral selector (4.6 x 250 mm; 5  $\mu$ m), by using a mixture of acetonitrile and water 65:35 v/v added with 0.1% formic acid. The nine pesticides (haloxyfop methyl, quizalofop, quizalofop-ethyl, fenoxaprop, fenoxaprop-ethyl, fluazyfop-butyl, penconazole, mandipropamid, pyriproxyfen) were extracted from river water and urine samples by means of graphitized carbon black-solid phase extraction (GCB-SPE) to examine the occurrence of potential residues and metabolites.

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**PESTICIDES IN SUPERFICIAL AND GROUND WATERS:  
METHOD DEVELOPMENT AND ANALYTICAL VALIDATION  
USING MULTIDIMENSIONAL GAS CHROMATOGRAPHY AND  
MASS SPECTROMETRY**

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The widespread use of pesticides for agricultural and nonagricultural purposes has resulted in the presence of their residues in surface and ground water resources.

Their presence in water is regulated through different directives, including the Ground Water Directive, the Drinking Water Directive, and the Water Framework Directive, modified later several times, setting a maximum concentration of 100 ng/L for individual pesticides and their degradation products, and 500 ng/L for total pesticide residues present in a sample.

For their determination and monitoring, multiresidue methods allow simultaneous determination of diverse classes of chemicals (e.g., organophosphorus and organochlorine pesticides, triazines, chloroacetamides, triazoles, (thio)carbamates, etc.) in a single analytical procedure.

In this contribution, more than 40 pesticides belonging to different chemical classes and their metabolites were selected based on their local occurrence and investigated in superficial and ground waters from agricultural areas susceptible to pesticide contamination.

The methodology used consisted of a classical solid-phase extraction (SPE) for the purification and enrichment of the pesticides, which were further analyzed and quantified using multidimensional gas chromatography together with mass spectrometry (GC×GC-MS). The method was also validated according to the Eurachem guidelines in terms of linearity, precision, accuracy, specificity, Limit-of-Detection and Limit-of-Quantification.

**HIGH-RESOLUTION FINGERPRINTING OF BALSAMIC VINEGARS USING MULTIDIMENSIONAL GAS CHROMATOGRAPHY AND MASS SPECTROMETRY**

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Balsamic vinegar is one of the best known and most popular types of vinegar. In Italy, and specifically in Emilia Romagna region, there are two protected designations of origin (PDO) and one protected geographical indication (PGI). These are exclusively produced at definite geographical areas (Modena and Reggio-Emilia) and with specific grape varieties grown for such a purpose.

The PDO traditional balsamic vinegars are obtained from cooked and concentrated must that is subjected to a dynamic aging process (in barrels of different types of woods and decreasing size) in which alcoholic and acetic fermentation take place simultaneously. The aging process can last at least for 12 years (*affinato*) or even 25 years (*extravecchio*).

The PGI is instead a more industrial product, and it often combines cooked wine must vinegar with caramel and other thickeners to obtain a similar density and flavor to those of the traditional product, but at a lower price to accommodate broader market needs. Here, a certain portion of cooked must is added to wine vinegar and then it is aged for a period that ranges up to 3 years in single wood barrels.

Aroma is a very important food feature to support its quality and to contribute to its acceptance. Aroma is determined by several hundreds of different volatile compounds that belong to different chemical families.

In this contribution, we exploited multidimensional gas chromatography together with mass spectrometry (GC×GC-MS) to characterize the composition of the volatile compounds and to associate it with the quality of these high-value products.



**UHPLC-QqQ-MS/MS FOR METABOLOMICS ANALYSIS OF FAECAL BILE ACIDS AND THEIR MAIN METABOLITES IN COLON DISEASE PATIENTS**

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Bile acids (BAs) are involved in several biological processes in the gut-liver axis including hepatotoxicity, intestinal inflammatory processes and cholesterol homeostasis mostly via modulation of farnesoid X receptor (FXR) signalling. Faecal Oxo-BA, stable intermediates of oxidation/epimerization reactions of the BA hydroxyls, could be relevant to investigate the crosstalk in liver-gut axis and the relationship between diseases and alterations in microbiota composition. With this aim, we developed and validated a new generation comprehensive and fast reversed phase ultra-performance liquid chromatography – triple quadrupole tandem mass spectrometry (RP-UHPLC-QqQ-MS/MS) method for the identification and analysis of up to 28 compounds including 21 Oxo-BAs and their metabolic precursors in human faeces. The method was validated by evaluating selectivity, linearity range, limit of quantification and detection, accuracy, precision, matrix effect and recovery. We compared the analytical performance of the new method with a previously developed method by UHPLC-QTOF-MS/MS [1], obtaining limits of quantification (LOQ) and detection (LOD) 5-fold lower. RP-UHPLC-QqQ-MS/MS method was then applied to the analysis of BA and their metabolites in faecal samples of control subjects and subjects presenting different colon diseases. Chemometrics tools, such as Principal component analysis (PCA), were used to data investigation, identifying variables able to discriminate between the different classes (i.e Oxo-LCA). We performed a linear discriminant analysis (LDA) to create a model able to discriminate the classes examined.

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**INVESTIGATION OF RETENTION BEHAVIOR OF NATURAL CANNABINOIDS ON DIFFERENTLY SUBSTITUTED POLYSACCHARIDE-BASED CHIRAL STATIONARY PHASES UNDER REVERSED-PHASE LIQUID CHROMATOGRAPHIC CONDITIONS**

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The growing popularity of cannabis products and recent legalization of cannabis for recreational purposes have contributed to the increase of the demand for analytical methods able to give a detailed characterization of cannabis samples and derivatives. In this context, one of the aspects that is strongly emerging is about the hazardous potential of uncharacterized minor cannabinoids, including chiral ones, for which achiral potency testing methods currently employed do not give any information. For this reason, there is a growing interest towards the development of liquid chromatographic methods for the enantioseparation of cannabinoids. Therefore, the retention behavior of five main cannabinoids (CBD, CBDA, CBC, (-)- $\Delta^9$ -THC and THCA) has been investigated on nine immobilised polysaccharide-based chiral stationary phases (CSPs) with different substituents, under reversed phase conditions. These nine columns are Chiralpak IA, IB-N, IC, ID, IE, IF, IG, IH and IJ. Results showed that chemo-selectivity of CSPs is not affected by changes in mobile phase composition, in the range of mobile phase investigated. In addition, the presence of electron withdrawing groups on the CSPs systematically leads to shorter retention times compared to when electron donating groups are present [1]. These findings suggest that polysaccharide-based columns are suitable for the analytical separation and quantification of the main cannabinoids present in real samples. Indeed, they can be employed for the characterization of cannabis products and derivatives also from the point of view of chirality.

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## ION-IMPRINTED POLYMERS AS A SUSTAINABLE STRATEGY FOR THE EFFICIENT RECOVERY OF LITHIUM IONS FROM WASTEWATER

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Lithium-Ion Batteries (LIB) are one of the most popular types of rechargeable batteries. Based on the current rate of lithium consumption, it is foreseeable that the demand for lithium salts will exceed global production from mineral sources no later than 2025. In this context, the recovery of lithium from spent LIBs is a mandatory alternative to primary geological sources. Currently, there is a lack of highly selective adsorption/ materials that can be used to recover lithium.

Ion-imprinted polymers (IIPs) have been increasingly developed during the last 10 years. They are pointing out on the principle of molecularly imprinted polymers (MIPs), but they specifically recognize metal ions [1]. This technique refers to an approach that involves preparation of macroporous and highly cross-linking polymers characterized by selective binding properties towards a target ion, previously introducing during polymerization and then accurately removed [2].

In this study, we present a development of a series of bulk-polymerization synthesis for IIPs, based on acrylate polyethylene glycol monomers knew to form a covalently stable pseudo-crown ethers in polymer mixtures. After template ions were completely removed from the IIP by using proper HCl solutions, the binding properties of IIP towards lithium (I) ion and potentially interfering ions such as sodium and potassium have been studied by equilibrium partition followed by ion chromatography analysis, to determine the binding parameters of the adsorption isotherms. To evaluate the imprinting factor and the non-specific binding, in parallel, a non-imprinted polymer (NIP) has been synthesized in the absence of the lithium (I) template. The results obtained were compared with the approaches currently available for lithium recovery.

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**AN ANALYTICAL METHOD BASED ON SOLID-PHASE EXTRACTION (SPE) COUPLED WITH GAS CHROMATOGRAPHY—ION TRAP/MASS SPECTROMETRY (GC-IT/MS) FOR THE EXTRACTION OF PHTHALATES (PAES) FROM THE BLOOD OF MARINE TURTLES.**

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The presence of phthalic acid esters (PAEs) in marine environments is an important issue. These chemicals are able to affect marine organisms, particularly marine turtles, and to act as endocrine disrupters. In this paper, for the first time, a simple and reproducible analytical method based on solid-phase extraction (SPE) coupled with gas chromatography—ion trap/mass spectrometry (GC-IT/MS) was developed for the extraction of phthalates from the blood of marine turtles. The extraction was obtained by using C18 phthalates-free as the stationary phase. In order to individuate the best working conditions for the extraction, the adsorption isotherms and breakthrough curves were studied. The overall analytical methodology was validated in terms of limit of detection (LOD, 0.08–0.6 ng mL<sup>-1</sup>), limit of quantification (LOQ, 0.4–0.8 ng mL<sup>-1</sup>), and correlation coefficients (>0.9933). By using this procedure, percentage recoveries ranging from 89 to 103% were achieved. The precision parameters (intra-day and inter-day) were studied, and the obtained values were smaller than 12.5%. These data confirm the goodness of the proposed analytical methodology, which is applied to real samples [1].

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**DEVELOPMENT OF AN ANALYTICAL METHOD BASED ON THE MICROWAVE-ASSISTED EXTRACTION AND SOLID-PHASE MICROEXTRACTION GAS CHROMATOGRAPHY-TRIPLE QUADRUPOLE MASS SPECTROMETRY ANALYSIS FOR THE DETERMINATION OF NITROPHENOLS IN INDOOR DUST**

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Humans spend a lot of their time in indoor environment and, therefore, the indoor dust is a good matrix of human exposure to organic pollutants. Nitrophenols are among the most important organic compounds and are listed as priority pollutants by the U.S. EPA. They can have various effects on human health because they show estrogenic, anti-androgenic and vasodilatory activity [1].

In the last years, there has been an increasing demand for new extraction techniques, amenable to reduce organic solvent consumption. In this context, microwave-assisted extraction (MAE) was a good choice for the extraction of organic compounds from solid matrices [2]. In this work a simple, efficient and environmentally friendly analytical method for the determination of 10 nitrophenols (NTPs) in indoor dust was proposed. For this purpose, a protocol based on the microwave-assisted extraction (MAE) of the analytes from the indoor dust followed by solid-phase microextraction gas chromatography-tandem mass spectrometry analysis (SPME-GC-MS/MS) was developed. Analytes were extracted using an environmentally friendly mixture and SPME was used to analyze the monitored compounds from the MAE extract by GC-MS/MS analysis eliminating additional sample clean-up steps. A derivatization step prior to the GC analysis was performed by testing several derivatizing reagents to obtain a selective and specific derivatization of the monitored analytes [3]. Experimental Design was used for the multivariate optimization of the parameters affecting the derivatization reaction and the MAE extraction. The signals were acquired in selected reaction monitoring (SRM) mode that allows the achievement of high specificity by selecting appropriate precursor-product ion couples.

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**METHODS FOR THE ANALYSIS OF THE PYROLYSIS PRODUCTS OF POLYETHYLENEIMINES BY GC-QMS, LC-ESI-MS AND LC-DAD**

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Polyethyleneimines (PEI) are versatile synthetic polymers obtained from the cationic polymerisation of aziridine. PEI can find use in a variety of present and future technological applications ranging from paper industry, household products, drug delivery systems and CCUS (carbon capture, utilisation and storage). In this latter field, PEI have been investigated as sorbents for trapping CO<sub>2</sub> in post-combustion processes and direct atmospheric capture. Despite the importance of this class of polymers, there has been a paucity of studies concerning their thermal degradation behaviour at a molecular level. In this contribution, analytical methods based on gas and liquid chromatography have been developed for the identification and quantitation of pyrolysis products of PEI.

Pyrolysis products of PEI were analysed by GC-QMS, LC-ESI-MS and LC-DAD. The pyrolysis products have been identified by EI-MS and ESI-MS and by comparison with pure standards. The principal constituents of the pyrolysates consisted of aliphatic acyclic oligomers, aliphatic cyclic amines (piperazines) and aromatic amines, principally pyrazine and alkylated pyrazines. In ESI-MS aliphatic amines produced quasi molecular ions, while pyrazines were poorly ionised. Pyrazines could be selectively analysed by reverse phase LC-DAD. Liquid-liquid extraction followed by GC-MS enabled the quantitative determination of pyrazines without the interference of aliphatic amines. After optimisation and performance evaluation, selected methods were applied to the analysis of samples obtained from the pyrolysis of PEI under different conditions. The results could be of interest in the framework of pyrolysis applied to the development of potentially valuable chemicals.

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**INVESTIGATION OF CANNABIS SATIVA L. VOLATILE FRACTION BY SIMULTANEOUS ENANTIOMERIC AND ISOTOPIC RATIO MEASUREMENTS**

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Recent times have witnessed an upsurge of interest in hemp and hemp-derived products, as driven by the scientific findings specific to the pharmacological properties of *Cannabis sativa* L. and its constituents. There has been evidence that the terpene profile, along with the cannabinoid content, produces in humans the effects associated with different strains, beyond fragrance perception. A great deal of effort has been put into developing analytical approaches to strengthen the scientific knowledge on cannabis essential oil composition and provide effective tools for ascertaining the authenticity of commercial cannabis samples. For this concern, enantio-selective-GC-C-IRMS has proven to be effective for assessing the ranges characteristic of the genuine samples and detecting any fraudulent additions. The study investigates the enantiomeric and isotopic ratios of target terpenes in cannabis essential oils, obtained from microwave-assisted hydro-distillation from the fresh and dried inflorescences of different cannabis varieties. Implementing multidimensional gas chromatography separation was mandatory prior to detection, in order to obtain accurate  $\delta^{13}\text{C}$  values and enantiomeric data from completely separated peaks. For this purpose, a heart-cut method was developed, based on the coupling of an apolar first dimension column to a secondary chiral cyclodextrin-based stationary phase. Afterwards, the data gathered from enantio-selective-MDGC-C-IRMS/qMS analysis of a set of genuine samples were used to evaluate the quality of commercial cannabis essential oils purchased from local stores. Remarkably, the data in some cases evidenced enantiomeric ratios and  $\delta^{13}\text{C}$  values outside the typical ranges of genuine oils. Such findings suggest the usefulness of the method developed to ascertain the genuineness and quality of cannabis essential oils.

**DEVELOPMENT OF A SOLID-PHASE TOXIN ADSORPTION (SPATT) METHOD BASED ON CROSS-LINKED CYCLODEXTRINS FOR MONITORING PALYTOXIN AND ITS CONGENERS**

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*Ostreopsis* cf. *ovata* is a benthic marine dinoflagellate whose proliferations have increased since the late 1990s, along the coasts of the Mediterranean Sea, including many Italian coastal areas, particularly along the Ligurian coasts.<sup>1</sup>The toxic effects are due to the presence of emerging toxins, palytoxin (PLTX) and its congeners (OVTXs), which are responsible for numerous human intoxications following inhalation of toxic aerosols, skin contact or ingestion of contaminated seafood. Considering that palytoxin is among the most potent non-protein marine toxins known to date, the necessity of an early warning of *Ostreopsis* proliferations turns out to be crucial to facilitate management and protect human health.<sup>2</sup>In this context, the Solid Phase Adsorption Toxin Tracking technology has been shown to provide reliable, sensitive, and time-integrated sampling of various aquatic toxins, to provide an early warning system for monitoring palytoxin and its congeners. In this work, cross-linked cyclodextrins (CDs) are proposed as new and sustainable materials for passive sampling of palytoxin and ovatoxins.<sup>3</sup>Several insoluble cyclodextrin polymers ( $\beta/\gamma$ -CD-hexamethylene diisocyanate ( $\beta/\gamma$ -CD-HDI),  $\beta/\gamma$ -CD-epichlorohydrin ( $\beta/\gamma$ -CD-EPI), and Diaion® as control) have been exposed to palytoxin standard solution and then with *O. cf. ovata* pellet extracts and suspension to evaluate the recovery yields of palytoxin and ovatoxins after being captured by cyclodextrins, extracted and finally analyzed by LC-HRMS. The preliminary results showed high recovery percentages for  $\gamma/\beta$ -CD-HDI and Diaion® as positive control, proving the efficiency of these materials for environmental monitoring.

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**CIRCULAR NONUNIFORM ELECTRIC FIELD GEL ELECTROPHORESIS FOR THE SEPARATION AND CONCENTRATION OF NANOPARTICLES**

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Efficient separation, purification, and concentration of micro-nanoscale objects are of great significance in various fields<sup>1</sup>. However, there are still some confronting problems such as complex matrices, low concentration of objects, and poor size uniformity, resulting in difficulty in achieving highly purified preparation and sensitive detection<sup>2,3</sup>. So, there is an urgent need to develop new techniques for the precise separation and efficient concentration of micro-nanoscale objects to satisfy the practical requirements. In this work, circular nonuniform electric field strategy coupled with gel electrophoresis was proposed to control the precise separation and efficient concentration of nano-microparticles. The circular nonuniform electric field has the feature of exponential increase in the electric field intensity along the radius, working with three functional zones of migration, acceleration, and concentration. The distribution form of electric field lines is regulated in functional zones to control the migration behaviors of particles for separation and concentration by altering the relative position of the ring electrode (outside) and rodlike electrode (inner). The circular nonuniform electric field promotes the target-type and high precision separation of nanoparticles based on the difference in charge-to-size ratio. The concentration multiple of nanoparticles is also controlled randomly with the alternation of radius, taking advantage of vertical extrusion and concentric converging of the migration path. This work provides a brand-new insight into the simultaneous separation and concentration of particles and is promising for developing a versatile tool for the separation and preparation of various samples instead of conventional methods.

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**MULTIWALLED CARBON NANOTUBES – CYCLODEXTRIN BASED COATING FOR ULTRASENSITIVE SOLID-PHASE MICROEXTRACTION GAS CHROMATOGRAPHY-MASS SPECTROMETRY DETERMINATION OF POLYCYCLIC AROMATIC COMPOUNDS IN ANTARCTICA ICE SAMPLES**

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The determination of polycyclic aromatic hydrocarbons (PAHs) in polar ice samples is very challenging because of the high sensitivity and selectivity required for their analysis at ultratrace levels, being recognized as indicators of anthropogenic contamination in samples collected in polar regions. Because concentrations are generally very low, analysis of PAHs with 4 or more aromatic rings in polar ice samples is particularly challenging, being these compounds more lipophilic than lighter PAHs [1]. Furthermore, to estimate the temporal resolution in the analysis of ice samples a significant reduction in the volume sample is required, so that miniaturized sample preparation techniques are demanded [2-4]. In this context, solid-phase microextraction (SPME) using nanocomposites based on multiwalled carbon nanotubes (MWCNTs) and  $\beta$ - and  $\gamma$ -cyclodextrins (CDs) was proposed to devise an innovative method for the extraction and GC-MS determination of 16 US-EPA PAHs in ice samples from Antarctica. Being characterized by great surface area and high adsorption capacity, MWCNTs have been successfully applied as SPME coatings [2,4]. The best extraction performance obtained by the MWCNT- $\gamma$ -CD fiber demonstrated the synergistic effect of  $\gamma$ -CD and MWCNTs in retaining PAHs, especially high molecular weight compounds. The MWCNT- $\gamma$ -CD coating resulted in GC-MS responses up to 6 times higher than those achieved by the non-functionalized nanotubes. Validation proved the reliability of the developed method for the ultratrace determination of the analytes, obtaining LOQ values in the low ng/L, good precision and trueness with RSD < 20% and recovery rates in the 88( $\pm$ 2)–119.8( $\pm$ 0.4)% range, respectively. Finally, both solid-state and  $^1\text{H-NMR}$  investigations confirmed the complexation capability of the  $\gamma$ -CDs towards the high molecular weight PAHs.

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**A NEW STATIONARY PHASE FOR CAPILLARY GAS CHROMATOGRAPHY BASED ON AMPHIPHILIC TRIBLOCK COPOLYMER - BENZIMIDAZOLIUM IONIC LIQUID**

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In this work, a novel amphiphilic triblock copolymer coupled with benzimidazolium ionic liquid (TCP-Bim) was designed, synthesized, and employed as capillary stationary phase for gas chromatography columns (GC). This substrate has been developed on the base of a previous study where new TCP column for gas-chromatography was realized and characterized[1]. The innovative TCP-Bim column exhibited moderate polarity and a column efficiency of 3280 plates/m, showing high resolutions for a wide range of analytes, with marked capabilities towards isomer separations, and different retention behaviors and resolutions respect to TCP or commercial HP-35 and PEG-20M columns. The new column has exhibited in some cases unmatched separation capabilities, like, for example, in the case of separation of chloroaniline and bromoaniline. These outstanding performances clearly indicate that TCP-Bim can be considered a very interesting candidate as a new type of stationary phase in GC.

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**DEVELOPMENT OF A COMPREHENSIVE LIQUID CHROMATOGRAPHY METHOD COUPLED TO TANDEM MASS SPECTROMETRY FOR THE DETERMINATION OF TARGET CONTAMINANTS IN CEREAL PRODUCTS**

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The present research focused on the development of a novel comprehensive liquid chromatography (LC×LC) method in order to enhance the chromatographic resolution and increase detectability for trace contaminants in cereal products. The latter were selected among vegetable products due to the ever increasing overuse of pesticides and fertilizer to maximize the crop yield. Specific contaminants were chosen due to their occurrence in cereals and because their maximum residue levels (MRLs) are strictly regulated by current legislation. Then, the purpose of the present study was to develop a robust, accurate and sensitive analytical method for their determination, in accordance with the above-mentioned MRLs and the validation criteria of the SANTE guideline document.

The LC×LC technique offers the advantage to remove interfering signals from the matrix, thus reducing limit of detection and quantification, and minimizing the need for laborious, tedious and time-consuming sample preparation steps, which could reduce the overall method accuracy.

Complementary stationary phases were selected in each chromatographic dimension to properly exploit different retention mechanisms and achieve satisfactory separation in the two-dimensional space. Targeted multicomponent analysis through time-segmented multiple reaction monitoring (MRM) were used to achieve high selectivity and sensitivity for quantification at low-levels.

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**CRYOGENIC TRAPPING APPLIED TO THE ISOLATION OF HIGHLY VOLATILE MOLECULES BY PREPARATIVE MDGC**

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A lab-made collection system capable to operate both under room temperature and cryogenic conditions was developed. The latter was exploited for the collection of volatile organic compounds by an efficient preparative-scale multidimensional gas chromatographic system. The study evaluated the collection performance of target analytes with a wide range of boiling points and different polarity. The prep-MDGC system consisted of three Deans switch transfer devices and a set of mega-bore columns. Such an approach assured the sample capacity looked for preparative purposes, overcoming the well-known low efficiency of wide-bore columns, through an orthogonal combination of stationary phases. The condensation of the analytes from the gas stream, after the chromatographic process, was optimized in relation to the different physicochemical properties of the target volatiles. As expected, specific collection conditions had a great influence on recovery values, depending on the different boiling points of the target compounds, especially for the highly volatile ones. Thanks to the prototypal system exploited in this study the critical points of Prep-GC applications were overcome. Additionally, the possibility to apply proper trapping strategies minimized the effect of compound specific properties over the collection performance achieving satisfactory recovery degrees.

## IDENTIFICATION OF CALENDULA AND JASMINE ESSENTIAL OILS BY GAS CHROMATOGRAPHYC ANALYSIS

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In recent decades, essential oil have gained interest not only for their aromatic properties, since research has focused on the therapeutic potential of plant-based extracts and essential oils. In fact, to date, the efficacy and safety of plant extracts against some pathogens are well documented. The study of terpenes compounds and their derivatives with potential health benefit in novel essential oils is continuously investigated. Essential oils produced with new green methodologies, which can increase their yield or improve their quality, or coming from different countries, and therefore considered innovative, are considered of great interest. In this regards, Mass spectrometry (MS) plays a fundamental role by providing a wide variety of methodologies both for the identification and structural characterization of the various molecules and for their quantitative determination.

In this research, the volatile profile of calendula and jasmine Egyptian essential oils has been investigated through GC-MS and GC-FID analysis.

Two different kind of calendula essential oil (concrete and absolute) have been analyzed in order to evaluate difference in composition and to correlate them with possible beneficial properties.

More than 170 compounds have been identified, belonging to different chemical classes: esters, alcohols, aldehydes, ketones, hydrocarbons and furans. For the identification FFNSC 4.0 and W11N17 spectral libraries have been used, applying the spectral similarity filter together with a Linear Retention Index (LRI) filter of  $\pm 10$  units.

**A DEEP INVESTIGATION OF VOLATILES IN MOSCATO GRAPES BY MEANS OF QUALI-QUANTITATIVE, ENANTIOMERIC AND ISOTOPIC DATA**

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Dealing with aromatic grapes, Moscato represents one of the most appreciated specie, due to the relative high amount of free and glycosidically bound terpenes, responsible for the typical aroma. In general, the high concentration of terpenols represents a common characteristic in Moscato specie, while their relative amount allow to distinguish between specific varieties. In this concern, from an analytical point of view, quali-quantitative analysis are usually performed by means of gas chromatography coupled to tandem mass spectrometry (GC-QqQ/MS) after a previous extraction step. However, beside quali-quantitative data, no information are reported in literature about isotopic and chiral data, although representing a valuable resource for genuineness assessment. In this concern, the development of a novel Es-GC-C-IRMS method may be considered useful to simultaneously determine both the enantiomeric and isotopic ratio of target terpenols. Nevertheless, such a monodimensional approach shows limitations when peaks co-elute. An optimized separation is in fact mandatory for both the revelation systems, even more for IRMS, due to the renowned chromatographic isotopic effect which would lead to unreliable results in case of peaks overlapping. To these aims, a novel enantiomeric multidimensional gas-chromatographic method coupled to isotopic ratio mass spectrometry was developed. Multidimensional conditions were thus optimized employing an apolar column in the <sup>1</sup>D and a chiral cyclodextrin based one in the <sup>2</sup>D. By these means, it was possible to simultaneously evaluate the enantiomeric and isotopic ratio of the main well separated terpenes in Moscato Giallo extracts, defining specific authenticity ranges.



**AUTHENTICITY ASSESSMENT OF VOLATILES IN AROMATIZED EXTRA VIRGIN OLIVE OILS BY MEANS OF ADVANCED ANALYTICAL APPROACHES**

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Recent times have witnessed an increased amount of healthy products on food market, having new catchy properties able to capture the interest of consumers. This is surely the case of aromatized extra-virgin olive oils (EVOOs). In relation to the price, these products are declared to be aromatized or by the maceration of natural matrices and olives or by adding natural flavouring to the EVOO. It's clear that such different preparation techniques involve in different economic values. Moreover, due to the high economic value of some flavourings, fraudulent practices may easily involve. To these aims, in the current study, various commercial aromatized EVOOs were investigated to evaluate the genuineness of the compounds responsible for the aroma, by employing different analytical approaches.

Dealing with sample preparation, head space solid phase micro extraction (HS-SPME) was chosen as the suitable analytical technique for the extraction of target volatiles, prior to gas chromatographic analysis. By means of GC analysis, quali-quantitative results allowed to compare experimental profiles with consistent literature data. However, for some aromatized EVOOs, as citrus aromatized ones, quantitative composition for the main volatiles may vary in relation to the seasonal production, reducing the possibility to discover adulterations. Moreover, since samples were prepared adding the "aroma" to olives, the resulting volatile profile was influenced from both the matrices. In this concern, for some aromatized EVOOs, additional analytical methods were developed along conventional HS-SPME-GC/MS approach. To these aims, chiral and carbon isotopic ( $\delta^{13}\text{C}$ ) analysis, preceded by multidimensional gas chromatographic separation, were led for target volatiles, comparing experimental results to consistent literature data.

**CIGUATERA FISH POISONING: LC-HRMS AND NEUROBLASTOMA CELL BASED ASSAY FOR THE BIOANALYTICAL IDENTIFICATION OF CIGUATOXINS IN *GAMBIERDISCUS BALECHII***

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Ciguatera Fish Poisoning (CFP) is one of the most worldwide spread food-borne illness due to the ingestion of fish and marine invertebrates contaminated by ciguatoxins (CTXs). CTXs are natural neurotoxins that act as voltage-gated sodium channels activators. They are produced by benthic marine dinoflagellates belonging to *Gambierdiscus* and *Fukuyoa* genera and are accumulated and bio-transformed in fish into more toxic congeners<sup>1</sup>. These dinoflagellates also produce gambieric acid and maitotoxins, which are bioactive toxic compounds<sup>2</sup>. The detection of CTXs in complex matrices (fish and shellfish tissues) at the very low concentrations at which they exert toxic effects (<1 ppb) requires advanced analytical methodologies and currently there are no validated routine tests to detect CTXs rapidly and reliably from the environment or in seafood. Therefore, marine toxin research is moving toward the discovery and structural characterization of new CTX congeners and the development of techniques for the chemical profiling and quantitation of compounds in toxin producing organisms. In this study, we describe a Liquid Chromatography High Resolution Mass Spectrometry (LC-HRMS<sup>n</sup>, n=1,2) method for the detection of CTX congeners. In lack of a sufficient amount of CTX reference material, the availability of a *Gambierdiscus balechii* culture and of a Neuroblastoma cell-based (N2a) assay for specifically testing CTX toxicity<sup>3</sup> played a key role in LC-HRMS method set-up. A bio-guided micro-fractionation approach revealed the presence of a putative I/C-CTX-1 or -2 and confirmed the presence of 44-methylgambierone in crude extract through chromatographic behaviour,

elemental compositions of HRMS ions, and interpretation of the fragmentation pattern.

<sup>1</sup>T. Pasinszki, J. Lako, T. E. Dennis, *Toxins* (2020), 12, 494

<sup>2</sup>S. Fraga, F. Rodriguez, P. Riobò, I. Bravo, *Harmful Algae* 58 (2016) 93–105

<sup>3</sup>C. R. Loeffler, D. Bodi, L. Tartaglione, C. Dell'Aversano, A. Preiss-Weigert, *Harmful Algae* 103 (2021) 101994

**HIGH PERFORMANCE LIQUID CHROMATOGRAPHY FOR THE IDENTIFICATION OF IMPURITIES IN DRUGS CANDIDATES**

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Active pharmaceutical ingredients (APIs) are the chemical-based compounds mainly used with combination of other ingredients to diagnose, cure, mitigate, and treat the disease [1]. During the synthetic process or during unsuitable storage of the drugs, a certain percentage of some related impurities can be produced, which are controlled by the official standard methods in order to ensure the efficacy and limit the toxicity of APIs [2]. In Europe, the *European Pharmacopoeia* (EP) defines references analytical methods, mostly based on High Performance Liquid Chromatography (HPLC), which have to be used to control the APIs quality before any pharmaceutical product manufacturing process. HPLC/PDA methods proposed in EP use chromatographic columns packed with totally porous particles. Normally, due to the low doses of APIs in pharmaceutical formulations, Chemical Reference Substance (CRS) are used to investigate the identity and proportion of impurities of pharmaceutical interest.

The objective of this study was to investigate the presence of APIs impurities by using EP analytical methodologies. Moreover, a comparative study between totally and partially porous particles was carried out in order to improve analytical method proposed by EP. For that purpose, salbutamol sulfate and bisoprolol fumarate were selected as a case study [3, 4].

[1] A. Kumar, V. Bansal, A. Madhavan, M. Kumar, R. Sindhu, M.K. Awasthi, P. Binod, S. Saran, *Bioengineered* 13 (2022) 4309-4327.

[2] E. Abbott, T.D. Veenstra, H.J. Issaq, *Journal of Chromatography A* 1382 (2015) 2-46.

[3] Salbutamol sulfate, monograph 07/2011:0687, EP 10.0

[4] Bisoprolol fumarate, monograph 01/2012:1710, EP 10.0

**AN ELECTROCHEMICAL BIOSENSOR FOR THE DETECTION OF MIRNA-29: ONE OF THE ACTORS INVOLVED IN THE PATHOGENESIS OF ALZHEIMER'S DISEASE**

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The development of electrochemical strips, as extremely powerful diagnostic tools, has received much attention in the field of sensor analysis. Nowadays, the detection of nucleic acids in complex matrices is a hot topic in the electroanalytical area, especially when directed toward the development of emerging technologies to facilitate personal health care.<sup>1</sup> One of the major diseases, for which early diagnosis is crucial, is represented by Alzheimer's disease (AD). The AD is a progressive neurodegenerative disease, and it is the most common cause of dementia worldwide. In this context, miRNAs (or microRNAs) are small noncoding RNAs that play an important role in the regulation of gene expression in eukaryotes. In particular, miRNA-29 represents a class of miRNAs known to regulate pathogenesis of AD.<sup>2</sup>

In this work we developed a strip, screen-printed on polyester support, able to detect two targets involved in the pathogenesis of AD, DNA and RNA sequences, thanks to the immobilization of an appropriate methylene blue (MB) probe on the electrode surface with gold nanoparticles (AuNPs) used to enhance the charge transfer kinetics at the electrode surface. The probe/target interactions have been characterized employing several biophysical methodologies. The system reached high sensitivity for both the target tested, with a 100 nM MB-probe immobilized on the electrode and a limit of detection of 0.6 nM for DNA Target in buffer solution and a lower affinity constant ( $K_a$ ) for the RNA target in standard solution and in serum matrix. The system has highlighted a satisfactory selectivity in presence of interfering sequences of both DNA and miRNA.

This work has the objective to open a way for the study and early diagnosis of a physically and socially devastating disease as Alzheimer's. The results demonstrate the suitability of this approach in terms of easiness to use, sensitivity, and applicability. The entire platform was conceived as disposable sensor, able to give rapid answers to patients, reducing the time gap between disease and treatment.

[1] S. Cinti, E. Proietti, F. Casotto, D. Moscone, F. Arduini, *Anal. Chem.* (2018), 90, 22, 13680-13686.

[2] S. Silvestro, P. Bramanti, E. Mazzon, *Int. J. Mol. Sci.* (2019) 20, 3979.

**EVALUATION OF REACTION AT THE INTERFACE SOLUTION/ELECTRODE BY ELECTROCHEMICAL AND SPECTROSCOPIC MEASUREMENTS**

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Coadsorption of different thiols is a very efficient approach to immobilize biorecognition elements in biosensors, using long chain thiols to anchor biomolecules and short chains as spacer, increasing both the functionalization yield and the performance of the final devices [1,2]. A peculiar and very promising application for mixed SAMs was reported in the gate electrode for biosensors based on electrolyte gated organic field-effect transistors (EGOFETs), able to reach the physical limit of sensitivity [3]. Macchia et al. also hypothesized a key role for SAMs in improving device performance thanks to the formation of a diffuse hydrogen bonding network between chains [3].

For this reason, cyclic voltammetry (CV), differential pulse voltammetry (DPV), and X-ray photoelectron spectroscopy (XPS) experiments was carried out to investigate SAMs conformational reorganization onto an electrode surface due to the application of an electrical field [4].

The results were compared with well-known trend available for other SAM structures for correlating supramolecular behaviour of the SAM due to the electric field application with performance of EGOFETs biosensors. [3,5]

[1] K. V. Gobi et al., *Anal. Bioanal. Chem.*, 387, 8, 2727–2735 (2007);

[2] S. Casalini et al., *Chem. Soc. Rev.*, 46, 1, 40–71, (2017);

[3] E. Macchia et al., *Nat. Commun.*, 9, 1, (2018);

[4] A. Tricase et al., *Nanomaterials*, 12, 5, (2022);

[5] A. Tricase et al., *Appl. Surf. Sci.*, 559, 149883 (2021)

## REORGANIZATION OF DNA-BASED POLYMERS FOR SENSING APPLICATIONS

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Non-covalent interactions are the cornerstone upon which Nature has built the mechanisms to achieve dynamic reconfiguration of biopolymers. Thanks to the high programmability of DNA/DNA and RNA/DNA interactions, synthetic nucleic acids can be used as self-assembling bricks to reconfigure nanodevices and nanostructures.<sup>1</sup> Over the past decade several strategies have been exploited to control the assembly of DNA-based nanostructures with biomolecular targets.<sup>2</sup> Motivated by this, we designed multiple DNA-based addressable building blocks (tiles) able to self-assemble into polymer-like structures that can be reorganized in presence of distinct RNA targets and RNA-degrading enzyme (Fig. 1).<sup>3</sup> The specificity of the involved non-covalent interactions allows to achieve a system with high orthogonality and programmability that could be extended to more complex structures and functions. This strategy paves the way for the design of reconfigurable nanostructures that can respond to specific markers (i.e. miRNA, lncRNA, circRNA) and therefore could have potential applications in sensing, diagnostic and therapeutic fields.

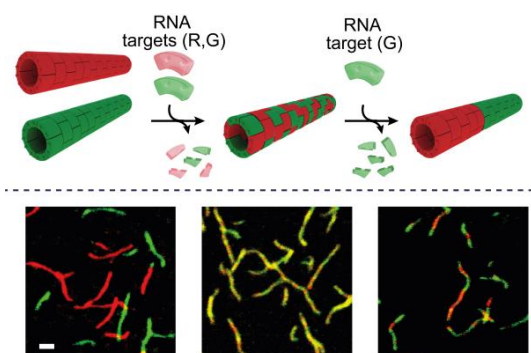


Figure 1. Reorganization of DNA-based polymers triggered by RNA targets.

[1] S. Gentile, E. Del Grosso, L. J. Prins, F. Ricci. *Anghew. Chem. Int. Ed.* 60 (2021) 12911-12917.

[2] K. E. Bujold, A. Lacroix, H. F. Sleiman, *Chem* 4 (2018) 495–521.

[3] S. Gentile, E. Del Grosso, P. E. Pungchai, E. Franco, L. J. Prins, F. Ricci *J. Am. Chem. Soc.* 143 (2021) 20296-20301.

## FUNCTIONALIZED GRAPHENE AEROGELS FOR GAS SENSING APPLICATIONS

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With the relevance of environmental issues, biomedical uses, and forensic applications, monitoring the concentration of volatile chemical compounds is one of the areas of greatest interest in scientific research. Materials with a two-dimensional nature, such as inorganic semiconductors, conjugated polymers and carbon nanomaterials have been explored for the gas sensing applications. 3D structures such as Aerogels have two major advantages, i.e., first, a high specific surface area and surface-to-volume ratio that provides sufficient surfaces for the adsorption of gas molecules; second, a 3D porous interconnected structural network that provides a stable and fast transport channel for the diffusion of gas molecules [1].

In the following work we explore the gas sensing behaviour of a covalently functionalized graphene aerogel, by monitoring changes in electric properties that occurs because of interaction with the analyte in the gas phase, making an overview on the different performances between two-dimensional and three-dimensional structures of graphene materials. To this end, we introduce a new method to covalently functionalize a reduced graphene oxide aerogel to improve the affinity between the probe and the analyte in the gas phase. To confirm the covalent functionalization, the new aerogel was characterized by spectroscopic techniques such as X-ray Photoelectron Spectroscopy (XPS), Attenuated Total Reflection-Fourier-Transform Infrared Spectroscopy (ATR-FTIR), and Thermogravimetric Analysis (TGA). Scanning Electron Microscopy (SEM) measurements were performed to evaluate the porosity of the aerogel sample.

[1] M.T. Noman, N. Amor, A. Ali, S. Petrik, R. Coufal, K. Adach, M. Fijalkowski, *Gels* (2021) 7, 264.



## 2D TRANSITION METAL DICHALCOGENIDES FROM EXFOLIATION IN WATER TO EFFECTIVE HETEROSTRUCTURES FOR SENSORS

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Two-dimensional transition metal dichalcogenides (TMDs) including MoS<sub>2</sub>, WS<sub>2</sub>, MoSe<sub>2</sub>, and WSe<sub>2</sub>, have received significant attention from the scientific community for their electronic, chemical, optical, and physical features. Despite this, their use in electrochemical sensors is often clouded by the needing for cumbersome procedures for their exfoliation to 2D structures, where solvent and dangerous/pollutant chemicals are needed. Moreover, since the TMDs' semiconductor nature, in the electroanalytical field, TMDs need to be combined with other materials to bring out their properties.

This presentation will be focused on the production of 2D-TMDs and carbon nanomaterials in water using natural compounds as functional 'surfactants', and their combination in 2D/1D and 2D/2D heterostructures using innovative approaches. To fulfill at the best the green chemistry principles, liquid-phase exfoliation/dispersion of the used nanomaterials have been conducted in water, taking advantage of functional bio and natural molecules as stabilizing agents.

Then Group VI TMDs have been used to form 2D/1D heterostructures with nanofibrillar biochar (BH) by direct water mixing; the heterostructures were used to modify SPE electrodes. BH proved to be an excellent support for TMDs, acting as a highly conductive hosting network; the electron transfer capacity of the BH was not affected by the presence of the semiconductors TMDs. On the other hand, the BH maximizes the catalytic features of the TMDs allowing improved electroanalytical performances. While reduced graphene oxide-based heterostructures including TMDs were fabricated by exploiting an innovative approach by using a CO<sub>2</sub> laser treatment. In this case, the formation of an effective 2D/2D heterostructure is also accompanied by a chemical change in the materials involved, which has led to a significant increase in the electrochemical performance of the films formed. The rGO/TMDs films have been employed to construct lab-made electrodes onto cellulosic substrates. The sensors integrating the proposed heterostructures demonstrated their potential in enzyme-free detection of different analytes including neurotransmitters, phenols, flavonols isomers, and hydrogen peroxide in model solutions and real samples. The obtained heterostructures allow to bring out TMDs' potentiality, becoming excellent candidates for the development of (bio)sensors and electrochemical devices through sustainable strategies and accessible technologies.

**SELF-CLEANING ZnO NANOSHEETS FOR PIEZOELECTRIC SENSORS**

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The design of wearable sensors coupling versatile analytical detection to self-cleaning is a highly desired combination, tackling the need of smart devices in response to the recent virus pandemics. To this aim, this work shows ZnO nanostructures obtained by a mild wet-chemistry approach onto ITO/PET flexible supports, resulting in wearable piezoelectric sensors exhibiting photocatalytic activity. ITO surfaces are treated with 0.5 mM KMnO<sub>4</sub> aqueous solution (20 minutes, 90°C); ZnO growth is subsequently carried out by a previously shown wet-chemistry method [1]. SEM analysis shows the presence of a good surface coverage of ZnO nanosheets (NSs) (about 1.5 NSs/μm<sup>2</sup>) in the case of treated ITO, whereas low-density larger flower-like ZnO NSs (about 0.25 NSs/μm<sup>2</sup>) are observed on the control ITO surface – i.e. not treated by the KMnO<sub>4</sub> solution. XPS analysis highlights the presence of zinc and oxygen, whereas manganese traces are detected by depth profile analysis. Chronoamperometric measurements show current reduction upon 1V bias towards bending, in full accordance with previous reports [2]. Electrical impedance spectroscopy (PBS buffer, pH = 7.4) shows that ZnO reduces impedance with respect to the bare ITO electrode at low sampling frequencies (0.1 -1 Hz), being ZnO NSs on treated ITO the system which provides the lowest values. In full accordance to the electrochemical data, piezophotocatalytic tests show that high-density ZnO NSs provide the best results towards photodegradation of methylene blue (25 μM, solar light simulator).

[1] G. Arrabito, V. Errico, Z. Zhang, W. Han, C. Falconi, 46 *Nano Energy* (2018) 54-62.

[2] M. Panth, B. Cook, M. Alamri, D. Ewing, A. Wilson, and J. Z. Wu, 5 *ACS Omega* (2020) 27359-27367.

**ELECTROCHEMICAL DNA-BASED PLATFORM FOR THE MULTIPLEX DETECTION OF CLINICALLY RELEVANT ANTIBODIES**

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The detection of specific antibodies plays a crucial role in the diagnosis of many diseases, infections and pathologies. Current methods for the detection of such biomarkers (i.e. ELISAs and western blots), remain cumbersome, reagent-intensive and multi-step processes so rapid, sensitive, and convenient platforms are urgently needed. In response to this, we have developed a rapid, user friendly, robust, sensitive and low-cost DNA-based electrochemical platform that take advantages from our recently reported antibody-controlled toehold mediated strand displacement reaction.<sup>[1]</sup> Specifically, our platform consists of synthetic nucleic acid strands two of which are conjugated to a recognition element (i.e. antigen) in order to achieve the release of a redox reporter-modified strand upon the recognition of a specific target antibody.<sup>[2]</sup> Our approach is sensitive, highly specific and selective enough to be employed in 90% serum. Due to the programmability of synthetic oligonucleotides, we have detected five different antibodies, three of which are clinically relevant (i.e. anti-HA, anti-HIV and Cetuximab antibodies) and we have achieved an orthogonal multiplexed detection with the use of different and compatible redox reporters. For all the above reasons, our platform may be useful in a wide range of applications, such as point-of-care diagnostics and antibody-based therapies.

[1] S. Ranallo, D. Sorrentino, F. Ricci, *Nat. Commun.* 10 (2019) 5509.

[2] S. Bracaglia, S. Ranallo, K.W. Plaxco, F. Ricci, *ACS Sens.* 6 (2021) 2442-2448.

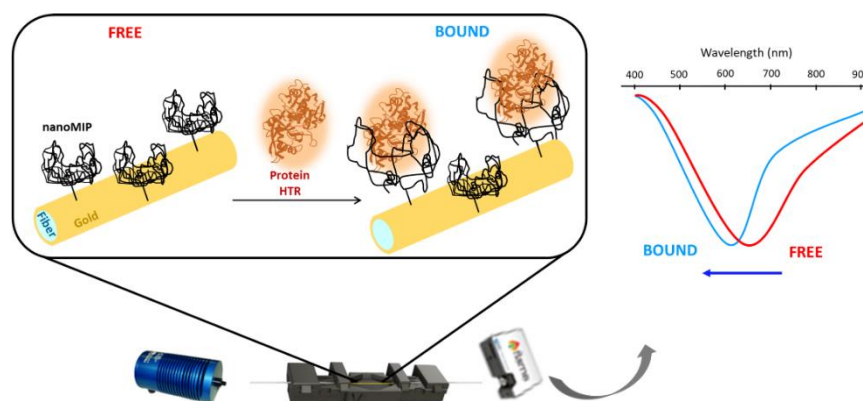
## SENSING OF PROTEINS THROUGH A PLASMONIC BIOSENSOR BASED ON LIGHT-DIFFUSING FIBERS FUNCTIONALIZED WITH MOLECULARLY IMPRINTED NANOPARTICLES

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Plasmonic bio/chemical sensing based on optical fibers combined with molecularly imprinted nanoparticles (nanoMIPs), which are polymeric receptors prepared by a template assisted synthesis, has been demonstrated as a powerful method to attain ultra-low detection limits, particularly when exploiting soft nanoMIPs, that are known to deform upon analyte binding [1]. This work, presents the development of a surface plasmon resonance (SPR) sensor in silica light-diffusing fibers (LDFs) functionalized with a specific nanoMIP receptor, entailed for the recognition of the protein human serum transferrin (HTR). Despite their great versatility, to date LDF-SPR were just reported functionalized with antibodies. Here, the innovative combination of a SPR-LDF platform and nanoMIPs led to the development of a sensor with an ultra-low limit of detection (LOD), equal to about 4 fM, and selective for its target analyte HTR. Worth to note, the SPR-LDF-nanoMIP sensor was mounted within a specially designed 3D-printed holder yielding to a measurement cell suitable for a rapid and reliable setup, easy for the scaling up of the measurements. [2]



**Figure 1.** Scheme of LDF-SPR-nanoMIP biosensor.

- [1] N. Cennamo, D. Maniglio, R. Tatti, L. Zeni, A. M. Bossi, *Biosens. Bioelectron.* (2020) 112126.  
 [2] F. Arcadio, M. Seggio, D. Del Prete, G. Buonanno, J. Mendes, L. Coelho, P. Jorge, L. Zeni, A. M. Bossi, N. Cennamo, *Nanomaterials.* (2022) 12.

**A PAPER-BASED SCREEN-PRINTED SENSOR FOR IRON DETECTION IN SERUM SAMPLES**

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The capability to perform analysis outside laboratory by non-trained personnel is gaining great relevance in the field of analytical chemistry. Following this research approach, in the last years, the development of point of care (POC) devices has been significantly increasing, especially for biomedical analysis, thanks to the possibility of enabling a rapid, user-friendly, and ubiquitous monitoring of people's health state.

Among the extensive plethora of point-of-care sensors, the ones exploiting electrochemical techniques deserve a primary role as they allow for the fabrication of deliverable, disposable, based on a minimal sample requirement and low-cost sensing tools.

Iron metabolism disorder is among the most common disease in humans and is a cause of severe clinical manifestations, e.g., anemia and iron overload.

The reference method for iron detection in serum is based on spectrophotometric detection, with several drawbacks, including a laboratory set-up, the need for high-volume samples, and skilled personnel, avoiding the possibility to perform point of care tests.

Herein we developed a miniaturized paper-based screen-printed sensor for iron detection in serum samples. First, the sensor was modified with Carbon Black and Gold Nanoparticles, and optimized to obtain best sensitivity and reproducibility. Several parameters of the square wave anodic stripping voltammetry were studied, namely deposition time, amplitude, frequency, and step potential. A Calibration curve was obtained in HCl 0.1 M, with a linear response in the range comprised between 0.05 and 10 ppm, a detection limit of 0.01 ppm and a regression equation equal to  $y = (0.8 \pm 0.4) + (2.66 \pm 0.09) x$  ( $R^2 = 0.996$ ). Finally, with the aim of performing iron quantification in real matrix, several human serum samples were tested. A treatment with acid solution (patent application # 102020000027546) and a centrifugation procedure were used to obtain the release of the bound iron ions and an accurate quantification. A comparison between the developed sensing tool with the reference method was performed, obtaining a good correlation within the experimental error.

**FOOD CONTAMINANTS DETECTION USING MOLECULARLY IMPRINTED BASED SENSORS: SYNTHESIS AND CHARACTERIZATION**

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Recently, molecular imprinted polymers (MIPs) played an important role in the development of reliable and stable sensors, considering the biomimicking nature of this platform with respect to several biorecognition elements like antibodies, DNA or enzymes, for instance enclosing nanomaterials as nanozymes.<sup>1,2</sup> MIPs are synthesised through a polymerization method to create semi-specific positions for targets in the molecular dimensions by using a monomer (*e.g.*, *o*-phenylenediamine, pyrrole *etc.*) and a target molecule.<sup>3</sup> In this work, we develop a MIP based electrode to sense dimethoate at ultralow/traces concentrations by using first electrochemical methods and later including this platform in an electrolyte-gated organic field effect transistor (EGOFET). At first, we characterised the modified platform by using several electrochemical techniques to demonstrate the effective removal of the templating molecule and the possibility to perform the rebinding/washing step multiple times, hence proving the stability of the prepared electrode surface. Furthermore, we performed a spectroscopic characterisation of the modified surface by means of X-Ray Photoelectron Spectroscopy (XPS) to prove the efficient template removal. Finally, the platform was also used to perform preliminary sensing measurements.

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## A SENSING APPROACH FOR THE MONITORING OF THE METALS RECOVERY IN RECYCLING BATTERIES

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As one of the iconic energy-storage technologies in modern-day society, lithium-ion batteries (LIBs) have cornered the energy storage market for portables like laptops, cell phones, cameras, to a variety of other electronic devices due to their high energy density for decades. In view of the environmental concerns globally, the consequences arising from the excessive emission of Greenhouse Gases like CO<sub>2</sub>, electric vehicles are considered as one promising solution. For this reason, the need of recycling automotive batteries had pushed scientists to find chemical methods for recovering the metals contained in these batteries and the monitoring of this recovery had become of outstanding importance [1]. Screen-printed electrodes (SPEs) are electrochemical sensors that are expected to be widely used in numerous real-life applications such as biology, medicine, environmental protection, and electronics [2]. This work presents the application of screen-printed electrodes for metal analysis in industrial wastes during the recycling process of batteries for automotive.

SPE modified with different metal nanostructures were applied for two purposes: i) adsorptive stripping voltammetric determination of nickel and cobalt ions by bismuth film and ii) potentiometric determination of lithium ions through a polymeric ion-selective membrane. The optimal conditions for the determination of nickel and cobalt in the form of dimethylglyoximate complexes, including the concentration of the complexing agent and the instrumental parameters, were selected. The optimal composition of the polymeric ion-selective membrane was also studied. The applicability of the developed platforms was assessed by determining lithium, nickel and cobalt in industrial wastes. These studies were complemented with a voltammetric characterization of the platforms by using the reversible redox couple ferrocyanide/ferricyanide.

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**IMMUNOTHERAPY ABUSE IN SPORTS: DEVELOPMENT OF A  
BIOSENSOR FOR MYOSTATIN INHIBITOR**

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Stamulumab (MYO-029) is a monoclonal recombinant human immunoglobulin G (IgG1) used for the immunotherapeutic treatment of muscular-wasting disorders, such as cachexia, sarcopenia, and muscle dystrophy. In detail, MYO-029 binds with a high affinity to myostatin and inhibits its activity [1]. For this reason, it is already suspected to be used to gain muscle mass for doping purpose, and it is included in WADA list of prohibited substances (S4-class: myostatin inhibitors) [2]. From the point of view of bioanalytical methods, at the present time, only classical immunochemical methods are available for the determination of immunotherapeutics, based on expansive and fragile capture antibodies. Accordingly, Stamulumab was used here as a case study to develop a novel diagnostic bioassay, based on a Surface Plasmon Resonance (SPR) sensing platform, through the production of catecholamine-based mimetic receptors, with the molecular imprinting technology. In this scenario, MIPs are proving to be very effective both in terms of technical performance and versatility, cost, and stability [3]. In addition, we have been exploring endogenous neurotransmitters as nature and sustainable building blocks (monomers) to straightforwardly design and synthesize a highly selective and sensitive MIPs. In details, we have been chiefly focusing on norepinephrine (NE) as functional monomer which is becoming increasingly appreciated as biocompatible material in accordance with its considerable advantage. Infact, polynorepinephrine (PNE) compared to polydopamine (PDA) shows lower hydrophobicity of surface, which minimizes unspecific binding and allows a fully reversible regeneration of the MIP surface [4]. The production of biomimetic receptor is relatively simple (by direct drop casting of the polymerizing NE solution on the SPR gold chip), cost-effective, fast (5h of polymerization) and does not require animal immunization. The strategy used for the protein imprinting is the so-called epitope imprinting approach, it is based on the use of a short “fingerprint” synthetic peptide (few amino acids 10-15 are rationally selected), as a template, that constitutes only a small portion of the large antibodies to which belongs, and that, in turn, may be recognized by the MIP. This technique allows achieving unexpected advancements (and reagents saving) in the recognition of large biomolecules, by mimicking the efficacy of the natural binding of antibodies. The results indicate the feasible use of imprinted PNE as synthetic receptor for MYO-029 and encouraging further investigations on potential application of this synthetic receptors for other immunoglobulin detection.



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**IMPEDIMETRIC SENSOR BASED ON ELECTROSYNTHESIZED MOLECULARLY IMPRINTED POLYMERIC FILMS FOR RELIABLE QUANTIFICATION OF D-GLUCONATE**

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D-gluconate is a product of the oxidation of D-glucose in metabolism of several microorganisms. It represents one of the predominant organic acids that can be found in vinegars, honey, and wines produced from grapes infected by *Botrytis cinerea* [1]. So, the quantification of D-gluconate in such matrices is of high interest in relation to product quality. The complexity of those matrices requires selective and accurate methods. Determination of D-gluconate in foodstuff (see e.g., in must [2]) is traditionally performed by separation techniques, but they are time-consuming, requiring tedious sample treatments to be performed. Enzyme biosensors have been used as suitable rapid alternative methods [3,4]. However, enzyme biosensors are based on expensive and fragile biological components which make them unsuitable for application in harsh environments. Their artificial counterparts, molecularly imprinted polymers (MIPs), possess unique features of selectivity, sensitivity, and stability also in complex matrices. These synthetic materials have attracted high interest since they are cheap, easy in preparation, which is also highly scalable. MIPs are prepared by polymerization of functional monomers interacting with the analyte molecules. After analyte removal, specific cavities are left as a sort of “memory” into the polymer network for further rebinding of the same molecules. Electrochemical preparation of MIPs can produce them as film directly attached to surface of electrodes in electrochemical sensors. In this work, we propose the development of an impedimetric sensor based on a MIP for D-gluconate. The synthesis of MIPs was performed by cyclic voltammetry of 3-aminophenylboronic acid onto the surface of screen-printed carbon electrodes (SPCE). Selection of the functional monomer was performed by molecular modelling. After template/analyte elution, electrochemical impedance spectroscopy (EIS) responses in the range of concentration between 1.9 and 250  $\mu\text{mol L}^{-1}$  in PBS (50 mM, pH = 7.8) were recorded on MIP and NIP (not imprinted polymer). Preliminary results on analytical performances will be reported.

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**NANOSIZED MOLECULARLY IMPRINTED PARTICLES INTEGRATED ON SCREEN-PRINTED PLATFORM FOR BIOMARKER SENSING. CASE OF TRYPSIN DETECTION**

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Since the onset of the COVID-19 pandemy, diagnostic tests for other clinical illnesses have drastically reduced. However, the early detection of other diseases in a situation where hospitals are busy remains of paramount importance. Therefore, the development of new analytical devices able to perform fast and accurate clinical screenings is crucial. Sensors are known to be promising for rapid screening activities; the specific development depends on the receptor architecture, nature of the analytes and the field of application. Recognition units with biomimetic ability are highly desirable to meet the demand of early detection of targets with high affinity and selectivity. Currently, the implementation of molecularly imprinted polymers (MIPs) has taken place in sensor development, thanks to their unique properties of selectivity, stability, ease of preparation and low cost. In the field of MIP, nanosized particles have been proposed for the imprinting process of large molecules of biological interest, such as proteins [1]. A relatively new approach, called “solid-phase synthesis”, can be easily performed in more compatible solvents (e.g., water) and it is fully automatable (ideal for large scale manufacturing, often a pivotal requirement in diagnostics). This protocol can be potentially used for diagnosis purposes to produce stable synthetic analogues of natural antibodies. A great number of proteins are currently used as therapeutic targets and disease biomarkers. Trypsin is an enzyme with anti-inflammatory properties, and it can be used as a biomarker for pancreatic cancer [2]. In this work, we propose an electrochemical immunosensor for the detection of trypsin. The sensor is based on water soluble nanosized MIPs (nanoMIPs) as receptor, immobilised onto screen-printed gold electrodes (SPAuE). Electrochemical characterisation by cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) confirmed the immobilisation of nanoMIPs on the electrode surfaces. EIS was employed to evaluate the sensor response towards trypsin, with concentrations between 1.4 and 97.6 ng/mL. A protocol for reproducible assay strategy will be proposed. Selectivity studies are currently in progress.

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**NOVEL ELECTRO-ANALYTICAL APPROACH FOR THE DETECTION AND DISCRIMINATION OF CANNABINOIDS IN REAL SAMPLES EXTRACTS**

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We have developed screen printed electrodes modified with carbon black (SPE-CBs) for qualitative and quantitative analysis of cannabinoids in different samples of cannabis. The electrochemical procedure proposed, together with the good detection performances of SPE-CBs, allow discrimination between different cannabis samples based on their cannabinoid content. Among phytocannabinoids the best known are  $\Delta^9$ -tetrahydrocannabinol (THC) and cannabidiol (CBD), even if the acidic precursors THCA and CBDA are mainly present in Cannabis sativa L samples. Marijuana, referred to as recreational cannabis, is illegal due to its high THC, which is psychoactive, and THCA content. Cannabis light products have recently become legal in many countries as they contain CBD and CBDA and a very low amount of THC. These types of products are growing exponentially, urging the need of an accurate control to meet specific regulations. The quantification of cannabinoids in real samples is currently performed by chromatographic techniques which require a consuming sample preparation, long analysis times and are not suitable for in-situ analysis. Cannabinoids are electroactive, due to the oxidation of the phenolic group so electrochemical sensors can be used as an alternative to chromatography. Moreover, these sensors have the advantages of being cheap, easy to handle and portable. In this work, we analysed extracts of different types of cannabis characterized by different cannabinoids contents. We investigated the sensor ability to recognize these samples by a multivariate approach, achieving good classification performances. On the other hand, we verified the possibility to quantify the THCA content in the different extracts using a calibration curve. Finally, we developed a prototype of a device capable of performing a rapid in-situ analysis of cannabis samples. This device uses micro-pumps and flow sensors to prepare all the solutions, starting from the extraction step, and finishing with the electrochemical analysis.

**DESIGN OF ALIZARIN BASED COLORIMETRIC WEARABLE SENSORS FOR ENVIRONMENTAL POLLUTANTS DETECTION**

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The most traditional methods for hazardous compounds detection in environmental samples, are represented mainly by high performance materials and techniques [1]. However, these methods require a not small consumption of time and money, as well as highly specific skills and methodologies from the operators, who carry out quantitative and qualitative determinations. Therefore, the possibility of rapid and *in situ* detection of hazardous compounds, as heavy metals in freshwaters, wastewaters or seas has attracted considerable interest in the research sector for the development of optical user-friendly wearable sensors that not require the use of sophisticated equipment, like colorimetric smart textiles. The main feature of a colorimetric smart textile is the presence of a bound dye (through a sol-gel matrix), characterized by a reactive centre that is able to strongly interact with the analyte [2]. On this regard, Alizarin red S (sodium 3,4-dihydroxy-9,10-dioxoanthracene-2-sulfonate) is widely used as a mordant dye, acid-base indicator, with applications in the biomedical and environmental field thanks to its capability of provide fluorescence or colour changes in the presence of some substances like carbohydrates, and different cations [3].

In this study cotton fabrics functionalized with alizarin-containing sol-gel coatings will be shown with an excellent durability of halochromic properties, thus emerging as a versatile platform for stimuli-responsive sensors, useful also for the colorimetric detection of heavy metals.

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**SCREEN-FIT: COMBINATION OF ELECTROCHEMICAL SCREENING STRATEGIES AND LC-MS CONFIRMATORY ANALYSES FOR PESTICIDES BIOMONITORING**

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Plant protection products are substances widely used to control pests such as insects, weeds, fungi and rodents. Most of these products enter in the human body by skin exposition, inhalation or ingestion with foodstuffs and cause neurotoxic effects, which could still be lethal. Due to the detrimental effects of pesticides for the human health, the dose-effect correlation becomes mandatory. However, at present, only few studies investigate pesticides concentrations in biological matrices such as blood, plasma, urine. This is because analyses are conducted with analytical methods that employ lab instrumentation, namely liquid chromatography coupled with mass spectrometry.

With SCREEN-FIT project, we aim at combining information coming from portable electrochemical sensors with mass-spectrometry methods, to develop a monitoring approach for plant protection products. Glyphosate (GLY) was considered by us as a model compound to test this approach. Several GLY detection platforms were reported in the literature, but their limited performance contributes to create the “*GLY paradox*”: GLY is the most applied herbicide on global scale [1] but it is also one of the most hardly determined [2]. To break this viscous cycle and to contribute in developing applicable methods for GLY monitoring, we compared several electrochemical sensing strategies designed for the direct and the indirect [3] electrochemical determination of GLY; the applicability of these platform was tested both in water standard solutions and in biological fluids, especially urines. This comparative study showed the limits and the potentialities of different sensing approaches showing their complementarity in terms of linear dynamic ranges and detection limits.

All samples analysed were screened also using confirmatory methods: a specific method for GLY detection in urines was developed to this aim.

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## AN ELECTROCHEMILUMINESCENCE APTASENSOR FOR $\beta$ -LACTOGLOBULIN DETECTION

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Food allergens can cause abnormal reactions of the immune system in hypersensitive subjects. About 90% of food allergies are linked to allergens contained in milk, eggs, fish, shellfish, tree nuts, peanuts, soy, and wheat, also referred to as Big-8. Cow's milk allergy is one of the most common food allergies in children with a prevalence of around 2.5%. Milk contains several allergens; the main ones are casein and  $\beta$ -Lactoglobulin ( $\beta$ -LG).  $\beta$ -LG is a protein relatively resistant to acid hydrolysis and protease activity. These characteristics allow it to preserve a certain structural integrity after digestion and to arrive intact in the intestinal mucosa where it elicits the immune response. At regulatory level,  $\beta$ -LG is not explicitly named. However, since milk is included in the list of 14 allergens present in Annex II of regulation 1169/2011, the presence of  $\beta$ -LG can be a useful marker for determining the presence of milk in food. Obtaining highly sensitive and effective sensors is crucial in the determination of allergens, since their presence in unlabelled products could reach levels that are of public health relevance. In this context, aptamer-based biosensors have greatly contributed to improve the quantification of allergens [1, 2], allowing the diffusion of simpler, cheaper and portable approaches. In this work, we present a portable and sensitive platform based on electrochemiluminescence (ECL) for the quantification of  $\beta$ -LG in food matrices. The proposed strategy employs luminol which is a widely used emitter in ECL. The performance and applicability of the sensor were tested by analyzing a sample of skimmed milk and an oat-based drink proposed as a vegetable substitute for milk of animal origin. We obtained a linear correlation between the intensity of the signal and the concentration of  $\beta$ -LG in the standard solutions ( $y = x [\mu\text{g L}^{-1}] * 0,00653 + 1,038$ ,  $R^2 = 0.99$ ). The limit of detection (LOD), and the limit of quantification (LOQ) were found to be 1.36 and 4.55  $\mu\text{g L}^{-1}$  of  $\beta$ -LG.

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**COST-EFFECTIVE AND PORTABLE 3D-PRINTED CARBON BLACK/THERMOPLASTIC POLYURETHANE ELECTROCHEMICAL SENSOR FOR THE DETECTION OF FREE CHLORINE IN WATER**

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Additive manufacturing has emerged as an innovative technology for the large-scale development of cost-effective and disposable electrochemical devices. Herein, we report the use of 3D-printed sensor using Carbon Black integrated Thermoplastic Polyurethane (CB-TPU) conductive filament for electrochemical detection of free chlorine, the most common chemical compound used for disinfection treatment of water [1]. This device is obtained exploiting 3D printing technique namely *Fused Deposition Modeling* directly from a computer aided design (CAD) data file. The process builds objects by adding material in a layer-by-layer fashion to create a three-dimensional (3D) part, offering the benefit to produce the complex parts with shorter cycle time and lower cost compared to traditional manufacturing process [2].

In this work, a simple arrangement of 3D-printed electrodes (working, reference, and auxiliary electrodes) was used. Carbon black dispersion was selected for working electrode surface modifier to exploit the properties of this nanomaterial to increase the electrochemical performances of the sensor [3]. No chemical and electrochemical procedures were applied for the pre-treatment of the electroactive surface. Under the optimized conditions (Britton-Robinson buffer 0.02 M + KCl 0.02 M, pH = 5, and applied potential of -0.1 V vs Ag / AgCl) a linearity up to 20 ppm was observed, described by the following equation  $y = (-0.128 \pm 0.006) x + (-0.38 \pm 0.06)$  with  $R^2 = 0.99$  with a LOD equal to 0.35 ppm. The 3D printed sensor was also tested in drinking water observing the absence of matrix effect. To evaluate the accuracy, three drinking water samples were tested both with the developed sensor and the spectrophotometric reference method (DPD), observing the agreement of the data.

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**DEVELOPMENT OF AN HYBRID ORIGAMI PAD ENZYMATIC BIOSENSOR FOR RELIABLE AND SENSITIVE DETECTION OF URIC ACID IN HUMAN SALIVA**

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Uric acid is the final product coming from human purine catabolism. It acts as a potent antioxidant against free radicals. Still, its dysregulated concentration in the human body might lead to clinical disorders like gout, Lesch-Nyhan syndrome, oxidative stress, etc.

Interestingly uric acid can represent an essential biomarker for the clinical diagnosis of hyper- or hypouricemia conditions [1]. In addition to the classical laborious and expensive diagnostic methods (e.g., spectral and chromatographic techniques) in the last decade, the scientific literature is a flourishing of works regarding electrochemical sensors for uric acid detection in human biological fluids, especially from serum [2]. Salivary diagnostics is recently emerging thanks to many biomarkers in human saliva, making it suitable for early diagnosis and monitoring of clinical disorders, thus avoiding the invasiveness of blood sampling [3]. In this work, we propose a hybrid biosensor device combining an origami filter paper pad and a polyester-based screen-printed electrode (SPE) for fast and non-invasive detection of uric acid from human saliva. The origami pad is used to entrap the uricase enzyme, and for the saliva sample collection, when uric acid gets in contact with the enzyme, hydrogen peroxide is formed and consequently detected when the pad is overlapped on the SPE. The detection is mediated by a Carbon black/Prussian blue dispersion used as a nano modifier of the working electrode to gain multiple advantages, among which are boosted electrochemical performances from carbon black and the possibility to work at shallow potentials. (i.e., -0,05V vs Ag/AgCl), thus avoiding current signal interferences thanks to Prussian Blue, which is known to be an “artificial peroxidase”. The developed origami hybrid biosensor resulted in a linear range broadening from 25 to 300 $\mu$ M, proving to be a potentially useful tool for an easy- and ready-to-use, eco-sustainable and non-invasive monitoring of uric acid from human saliva.

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## **ELECTROCHEMICAL PLATFORM FOR ARA H1 PEANUT ALLERGEN DETECTION**

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Ara H1 allergie is considered one of the most severe, life-threatening food sensitivities since it triggers the highest frequency of severe and fatal reactions, even in trace amounts. Therefore, it is extremely important to develop fast, accurate and easy-to-use analytical methods to determine Ara h1 allergen from food products that might contain traces of peanuts.

Here, we present the results obtained during the development of an electrochemical aptasensor based on screen-printed carbon electrodes (SPCEs) modified by graphene oxide with carboxylic groups (GO-COOH) and metallic nanoparticles for ARA H1 detection. Thanks to the affinity reaction between the specific DNA receptor and the target analyte, the aptasensor showed high specificity and good sensitivity towards Ara H1.

The aptasensor was applied in the linear range between 0 - 150 nM, and a limit of detection of 1.66 nM was obtained. The method was applied in peanut-free food samples with very good recoveries proving to be a promising tool for peanut allergy prevention.

### **Acknowledgments**

The authors acknowledge the financial support of Italian Government in the framework of PRIN 2017, Prot. 2017YER72K\_005 and Romanian Ministry of Education and Research, CNCS-UEFISCDI, project number PN-III-P1-1.1-PD-2019-0631, within PNCDI III.

**ENTROPY-BASED RATIONAL MODULATION OF STIMULI-RESPONSIVE PROPERTIES OF SYNTHETIC DNA NANOSWITCHES**

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Herein, we present a generalizable and versatile strategy to program stimuli-responsive properties of synthetic DNA nanoswitches in which the capacity of the synthetic receptor to respond a specific ligand or an environmental input (e.g., pH or temperature) can be finely modulated by controlling the entropy associated with the linker connecting the ligand-binding or pH-responsive domains.<sup>[1,2]</sup> To do so, we have re-engineered two different model DNA-based receptors and a set of pH-responsive nanoswitches. Specifically, as receptors we used a triplex clamp DNA-based receptor that recognizes a specific DNA sequence and an ATP-binding aptamer; and as pH-responsive nanoswitches we exploited the formation of an intramolecular triplex structure through hydrogen bonds (Hoogsteen interactions) between a hairpin duplex domain and a single-strand triplex-forming domain. We show that, by varying the length of the linker domain that connects the two ligand-binding or pH-responsive domains of these receptors, it is possible to: 1) finely control their affinity for their specific ligand;<sup>[1]</sup> 2) modulate their observed acidic constant ( $pK_a$ ), therefore, their pH-dependence;<sup>[2]</sup> 3) program their thermos-responsive properties in order to release a specific molecular ligand at a defined temperature. Through mathematical modelling, thermodynamic and kinetic characterization, we demonstrate that the modulation of stimuli-responsive properties of the nanoswitches results dependent on the total entropy associated with changes in linker length. Furthermore, the length of the linker does not affect the efficiency of the receptors during their loading/release process or binding. The possibility to rationally design stimuli-responsive DNA nanoswitches using purely entropic domains can be of utility in applications such as biosensing, drug delivery and production of smart materials in which the modulation of these systems could be obtained through a versatile, precise, predictable, and tunable approach.

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## ACTIVE Ag-NANOPARTICLES FOR HEAVY METAL IONS DETECTION.

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Heavy metal pollution is a serious global environmental problem due to the adverse effects it is causing around the world. Therefore, a great deal of interest has developed versus sensors that allow to detect heavy metals using user-friendly and sensitive way. Colorimetric sensor-based on gold or silver nanoparticles (NPs) has increasingly been used thanks to their unique properties such as Localized Surface Plasmon Resonance (LSPR); this result in a strong absorption band in the visible region of the spectrum, depending on the size and shape of NPs and on dielectric constant of the medium. The most popular colorimetric sensor approach is based on metal nanoparticle aggregation [1]. In fact, a decrease in the interparticle distance, induced by the NPs assembly, change the plasmonic properties. To improve selectivity of this type of sensors, the NPs can be functionalized with ligands containing at least two functional groups, the tail that interact with the surface of particles, and the head that interact with the target molecules promoting the NPs aggregation [2].

In this study, the effect of functionalizing molecule (FM) density, on the variation of Surface Plasmon Absorption Band (SPAB) of AgNPs@FM induced by the addition of metal ions was investigated. The study demonstrated as to different FM concentration corresponded a particular AgNPs@FM behavior and therefore a definite shift of the SPAB. AgNPs@FM with different FM density were prepared and titrated by consecutively addition of metal ions (M); calibrations curves for each metal ions were obtained. It has been observed that, with a specific FM density, a second SPAB appeared at the wavelength related to each metal ions. Interestingly, different colors, related to different superlattice of AgNPs@FM@M were obtained allowing the identification of each metal ions. This preliminary investigation allows to understand how tuning FM density on the AgNPs allow to modulate the sensor response.

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**A HYBRID NANOCOMPOSITE BASED ON NANO-GRAPHENE OXIDE DECORATED WITH AU NANOPARTICLES FOR THE ELECTROCHEMICAL DETERMINATION OF ESTRONE**

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A hybrid nanocomposite formed of nano-graphene oxide (nGO) flakes decorated with gold nanoparticles (AuNPs) was used to modify disposable screen-printed carbon electrodes (SPCE). Compared to conventional carbon-based sensors, such surface modification was able to improve the electrocatalytic effect, as well as the electron transfer rate, the electrochemical efficiency, and the overall sensitivity of the platform. Herein, we have focused on nGO/AuNPs synthesised with different nGO: Au w/w ratios, characterising them electrochemically in the presence of redox probes. Furthermore, such nGO/AuNPs-modified sensors were applied for the detection of estrone, as a contaminant of environmental interest, by designing an immunomagnetic assay.

*Acknowledgements*

Financial support from Regione Toscana Bando Salute 2018 (Research project CUP n. D78D20000870002) is acknowledged.

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**HYDROGEN PEROXIDE DETERMINED IN MILK AND COSMETICS USING NEW LDH-CLARK-TYPE-CATALASE BIOSENSOR.**

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Recently, we presented a contribution concerning the realization of a new biosensor system, for H<sub>2</sub>O<sub>2</sub> analysis [1], based on a glassy carbon (GC) electrode modified with Layered double hydroxide (LDH) of the type [Zn<sup>II</sup> Al<sup>III</sup> (OH)<sub>2</sub>]<sup>+</sup> NO<sub>3</sub><sup>-</sup>, with the addition of an enzyme cross-linked in the LDH matrix, which was strongly fixed on the GC by silver paste and cross-linking with glutaraldehyde vapours. Main virtue of new device seems to be a very long duration, not less than at least two and a half months, in operative conditions. The limit of detection (LOD) of the enzyme catalase biosensor for hydrogen peroxide was about 0.20 mM. However, similarly to all other modified GC enzyme sensors of the same kind, the major drawback of this biosensor, which greatly limits its application, is the high sensitivity to possible interferences that may occur in the analysis of real samples, which show turbidity or emulsions, or containing species that can interact with the working electrode, for instance if strong oxidizing species, are present. To overcome this limitation, in the present study, a different type of catalase biosensor was built, which still makes use of adsorbed catalase enzyme on LDH, but that no longer uses a GC electrode, being based instead on an amperometric Clark-type transducer, i.e., an amperometric sensor with a gas permeable membrane coupled to a platinum working electrode. This new biosensor configuration allowed avoiding all interferences, caused both by turbidity or emulsion, and especially interacting with redox water-soluble species. In the case of the present research, it also allowed the determination of H<sub>2</sub>O<sub>2</sub> in some real matrices, such as milk and common cosmetic preparations. These matrices are very difficult to analyze in simple, fast and inexpensive way, because they usually contain very low concentrations of hydrogen peroxide and are also cloudy, so that they are impossible to be analyzed with a simple GC-LDH biosensor. An inaccuracy ≤ 7.7% for cosmetic samples and ≤ 8.0% for milk samples and a precision between 0.7 and 1.5 (as RSD%), depending on the cosmetic or milk samples analyzed, were achieved using the new LDH-Clark type biosensor.

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**PALLADIUM COMPLEXES ON 2D CARBON FOR LOW HYDROGEN PEROXIDE PRODUCTION IN THE OXYGEN REDUCTION REACTION**

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Oxygen Reduction Reaction (ORR) is the bottle-neck strategic reaction ruling the fuel cell efficiency process. This reaction can take place in two main ways: from a direct reduction to water, with the exchange of 4 electrons per oxygen molecule or from an intermediate reduction to hydrogen peroxide, with the exchange of two electrons. Analytical quantification of the amount of hydrogen peroxide produced during the reaction is of fundamental relevance to the evaluation of the catalytic activity of electrocatalysts. Indeed, hydrogen peroxide is an undesirable corrosive product that reduces the efficiency of alkaline fuel cells. The best available catalysts which prevent the formation of hydrogen peroxide belong to the so-called platinum group metals (PGM). PGM are very expensive, hence lowering the PGM content of ORR catalysts is among the most prevalent research focuses [1]. In this work we propose Single-Ion catalysts formed by carbon nanotubes functionalized with Pd(II) complexes showed surprising catalytic activity [2] and a low production of hydrogen peroxide. Oxygen Reduction Reaction performance is evaluated via rotating ring-disk electrode (RRDE) and rotating disk electrode (RDE) which are the two best techniques for assessing the amount of hydrogen peroxide formed during the ORR. Results confirm that materials tested catalyse the four-electron reaction pathway and reduce the formation of hydrogen peroxide. These approaches provide the basis for further developments to improve the efficiency of future low-cost catalysts for alkaline fuel cells.

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**ELECTROREDUCTION OF ORGANIC COMPOUNDS: THE HALOGENATED NAPHTHALENE DERIVATES CASE STUDY**

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The electroreductive cleavage of the carbon-halogen bond, in halogenated organic compounds, is a widely studied topic [1]. Since the possibility of removing the halogen group from organic substrates has been considered for environmental applications [2]. Indeed, several organic halocompounds are known to have an environmental impact due to their use as herbicides, or cleaning agent. The electroreduction mechanism of 1-Br-2-naphtol, 1-I-2-naphtol and 1-Br-2-methylnaphalene is characterized by means of an integrated electrochemical and theoretical approach. The results of an exhaustive electrochemical characterization based on cyclic voltammetry measurements are presented and integrated with the outcome of complementary chromatographic analytical techniques: the electro-reduction products were determined and characterised by LC-DAD and LC-MS. The carbon-halogen bond dissociation is disassembled at a molecular level exploiting quantum mechanical calculation. Steady state, equilibrium, and dissociation dynamics, via molecular dynamics (MD) DRC calculations are performed, to achieve a detailed picture of the dissociation process. Experimental and theoretical results, indicate that the carbon-halogen bond dissociates following the first electron uptake, with a stepwise mechanism. The present study was supported by the MIUR-Italy (“Progetto Di-partimenti di Eccellenza 2018–2022” allocated to the Department of Chemistry “Ugo Schiff” of the University of Firenze, Italy).

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## HALOACETIC ACIDS DETECTION BY ELECTROCHEMICAL SENSORS

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The WHO Guidelines for drinking-water quality and the EU Directive 2020/2184 recommend the use of Water Safety Plans for the operational monitoring of many parameters in the distribution system [1,2]. The development of sensors for disinfection by-products, as haloacetic acids (HAA), represents one of the actions for water monitoring strategies. HAA are considered Contaminants of Emerging Concern and they include monochloroacetic, dichloroacetic, trichloroacetic, monobromoacetic, and dibromoacetic acid.

According to literature, silver nanoparticles (SNP) catalyze the reduction of Cl and Br moieties of HAA compounds [3,4]. In this study, electrochemical sensors for HAA detection have been prepared by decorating Glassy Carbon Electrodes (GCEs) with SNP. Scanning Electron Microscopy (SEM) measurements confirmed the SNP formation on GCE surface, while CV measurements let assess faradic current obtained from modified electrodes with respect to the bare one. Particularly, CV signals evidenced reduction peaks characteristic of the target compound.

The electrocatalytic properties of SNP for the electrochemical detection of HAA were also explored in combination with innovative graphene derivatives to take advantage of the adsorption properties of this nanosized materials; pristine reduced graphene oxide and relevant derivatives possessing tailored functionalization were tested aiming at increasing the sensitivity for the detection of the target analytes. Quantification of different HAA was achieved by pulsed voltammetric methods, to define LOD, LOQ, sensitivity and linear interval.

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**ELECTROANALYTICAL METHODS FOR QUANTIFYING THE CONTENT OF ORGANIC ADDITIVES IN COMPLEX MATRICES OF COPPER PLATING BATHS**

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Commercial plating baths may contain a great number of organic additives that play an important role in assuring the quality of the deposits. During the electrodeposition process these compounds tend to wear out and degrade so, they must be frequently monitored and dosed to keep constant their concentration and do not alter the technical and aesthetic proprieties of the deposits. Since plating baths are complex matrices where organic additives are present in minimal quantities respect to the other components, electrochemical methods represent a reliable alternative to the conventional chromatographic techniques because they make it possible to carry out quantitative analyses taking into account the effects of additives on the electrodeposition rate regardless of their chemical nature and providing, at the same time, useful information about the condition of the bath [1]. Cyclic Voltammetric Stripping (CVS) measurements were carried out on a commercial acid copper plating bath to determine the concentrations of the three main additives generally used in these baths. Various analytical methods, such as dilution titration and standard additions, have been selected depending on the additive under investigation. The experiments were performed by using a systematic approach, varying the different parameters involved. Electrochemical Impedance Spectroscopy (EIS) experiments were also conducted to characterize the deposition process in terms of resistive and capacitive components. The application of EIS in the galvanic field is of great interest both from an industrial and academic point of view, to achieve a detailed knowledge of the function of each additive and the interactions between them and to study the aging of plating baths. The authors acknowledge Regione Toscana POR CreO FESR 2014-2020 – azione 1.1.5 sub-azione a1 – Bando 1 “Progetti Strategici di ricerca e sviluppo” which made possible the projects “A.C.A.L. 4.0” and “GoodGalv” and MUR and EU-FSE for financial support of the PhD fellowship PON Research and Innovation 2014-2020 (D.M 1061/2021) XXXVII Cycle in Chemical Sciences.

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**NICKEL RELEASE: THE UNEXPECTED BEHAVIOR OF ELECTROPLATED STAINLESS STEEL**

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Steel is one of the most important materials in the world because, besides being relatively inexpensive, it can be continuously recycled without losing its technical properties. Nevertheless, its presence in the electroplating sector is very limited due to the difficulties involved in electroplating. Stainless steel (316L) contains approximately 10.5% chromium, which forms a surface oxide layer that passivates the steel <sup>[1]</sup>, making it resistant to corrosion and difficult to electroplate.

The main method of electroplating stainless steel is generally through the deposition of nickel, but a strict regulation limits its use due to the issues related to this metal. Indeed, nickel allergy is the most frequent contact allergy in the world, affecting 10–15% of women and a few % of men in the general population <sup>[2]</sup>. Even when nickel-free processes are used, it is possible that steel objects release nickel beyond the threshold value allowed by current legislation. This is because the steel itself contains nickel (ca. 12% in 316L), so the removal of the surface oxide layer during the electroplating process may facilitate the release of nickel.

The aim of this work is to develop a new plating process to avoid the Ni release from stainless steel and understand the mechanism behind this phenomenon by means of analytical techniques, such as SEM-EDS, XRF and ICP-MS. In a world that needs a transition to industrial processes that have a low impact on the environment, the electroplating industry wants to play an active role in helping to improve the sustainability and eco-friendliness of its processes.

The authors acknowledge Regione Toscana POR CreO FESR 2014-2020 – azione 1.1.5 sub-azione a1 – Bando 1 “Progetti Strategici di ricerca e sviluppo” which made possible the project “A.C.A.L. 4.0” (CUP 3553.04032020.158000165\_1385).

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## **ELECTROCHEMICAL PLATFORM FOR ARA H1 PEANUT ALLERGEN DETECTION**

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Ara H1 allergie is considered one of the most severe, life-threatening food sensitivities since it triggers the highest frequency of severe and fatal reactions, even in trace amounts. Therefore, it is extremely important to develop fast, accurate and easy-to-use analytical methods to determine Ara h1 allergen from food products that might contain traces of peanuts.

Here, we present the results obtained during the development of an electrochemical aptasensor based on screen-printed carbon electrodes (SPCEs) modified by graphene oxide with carboxylic groups (GO-COOH) and metallic nanoparticles for ARA H1 detection. Thanks to the affinity reaction between the specific DNA receptor and the target analyte, the aptasensor showed high specificity and good sensitivity towards Ara H1.

The aptasensor was applied in the linear range between 0 - 150 nM, and a limit of detection of 1.66 nM was obtained. The method was applied in peanut-free food samples with very good recoveries proving to be a promising tool for peanut allergy prevention.

### **Acknowledgments**

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## A PRELIMINARY VOLTAMMETRIC STUDY OF SILVER IONS IN LIPOPHILIC FOOD SIMULANT

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Recently, nanomaterials (NMs) have gained increasing popularity in the food industry, particularly for product preservation and food packaging, as their use can improve the thermal and mechanical resistance of products and, above all, imparts antimicrobial properties to packaging [1]. In this context, silver nanoparticles occupy a primary place thanks to their well-known activity, mainly attributed to ionic silver form, against a wide variety of both Gram-positive and Gram-negative bacteria. However, silver species, especially soluble Ag(I) ions, could be released from such products into food matrices and this could pose risks to human health. Therefore, studies concerning their migration from food packaging to the external environment have become necessary.

Investigation on Ag(I) release typically involves contacting the test materials with fluids that simulate the physico-chemical properties of food, and then detecting the amount of Ag species released. According to the European Commission, for aqueous and semi-fatty foods, suitable fluids as food simulants are water solutions and water/ethanol mixtures, while, for fatty products, vegetable oils are indicated as lipophilic simulants [1].

Quantification of silver species released in food simulants are in general performed by spectroscopic techniques, providing total silver detection. Instead, to distinguish between ionic silver from other forms in liquid media, electrochemical approaches are more suited. In the literature, only a few voltammetric investigations on Ag(I) ions release in food simulants with a high-water content have been reported, while, to the best of our knowledge, no report exists on the migration of Ag(I) ions in lipophilic food simulants.

Accordingly, in this study we investigated on the voltammetric behavior of Ag(I) ions in a lipophilic simulant made by olive oil mixed with the ionic liquid [P<sub>14,6,6,6</sub>][NTf<sub>2</sub>], which is known to be soluble in vegetable oils and act as supporting electrolyte [2]. Sets of measurements were performed, using a microdisk electrode, at temperatures ranging from 25 to 40 °C, *i.e.* the interval normally used for migration tests of species from food packaging. Under these conditions, the diffusive parameters of Ag(I) ions were studied to define mass transport characteristics of the species in the test media, as they are fundamental for the definition of analytical quantification protocols.

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## HYDROGEL-FILLED MICROPIPETTE. A NEW TOOL FOR ELECTROANALYTICAL INVESTIGATIONS AT INTERFACES

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This paper reports on the use of a novel microcapillary system for solid contact electrochemical measurements. The probe is made of moveable micropipettes, with orifice of 1-30  $\mu\text{m}$  radii, filled with a conducting hydrogel, which forms a thin-gelled meniscus at the pipette end. The hydrogel is made of 2 % (w/v) agarose and water solutions, containing KCl or  $\text{KNO}_3$  as supporting electrolytes. The micropipette can be brought in contact with a conducting substrate to form a microcell, which allows performing voltammetric measurements confined within limited contact regions (Figure 1a). The suitability of the proposed probe for local electrochemical measurements are tested using two electroactive species, dissolved in the hydrogel, namely  $[\text{Fe}(\text{CN})_6]^{4-}$  and  $\text{Ag}^+$  ions (Figure 1b). Mass transport characteristics of the two species, in bulk hydrogel and at micropipette menisci of different radii, are examined in detail in the frame of existing theory. For comparison, voltammetric measurements are also performed with micropipettes filled with the corresponding aqueous solutions. It is shown that the gel-filled micropipette, at variance with the aqueous one, prevents the spreading and leakage of solution on the sample surface. The microprobe developed here can be useful to perform electrochemical measurements on surfaces, which suffer from direct contact with liquid electrolytes. A Proof-of-concept hydrogel-capillary measurement is performed to distinguish the presence of metallic silver deposited on a graphite-on-paper based material, realized through simple pencil strokes (Figure 1c).

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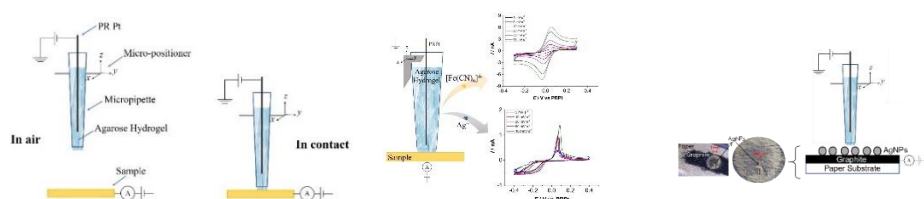


Figure: a) Scheme of the micropipette filled with agarose gel and working principle; b) typical voltammetric responses; c) recognition of AgNPs on an on-paper based material.

**SPECTROSCOPIC CHARACTERIZATION OF INDUSTRIAL SUBSTRATES TREATED BY ANTIMICROBIAL COATINGS**

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Industrial substrates like leather, leatherette, polycarbonate, polyurethane, etc., modified by bioactive coatings represent one of the outcomes of the Italian MIUR project “E-Design” ARS01\_01158. Antimicrobial functionality is ensured by a proper choice of metal nanoparticles (MeNPs), such as silver, copper and zinc oxide, which are embedded in polymeric matrices. Stabilized MeNPs are able to release active ions, under defined kinetics, that inhibit bacterial growth [1-3]. Different antimicrobial materials were successfully applied as active components of coatings to be used to modify several industrial goods for the automotive industry.

In this contribution, the most representative results of the analytical characterization of the composite materials developed in our laboratories will be presented. Scanning Electron Microscopy (SEM) was used to assess the composite morphology. X-ray Photoelectron Spectroscopy (XPS) provided quantitative information about metal surface availability, and surface chemical state. Electro-Thermal Atomic Absorption Spectroscopy allowed investigating metal ion release in aqueous contact media. The potential release of entire nanoparticles from treated materials was studied by Transmission Electron Microscopy (TEM) on contact solutions. Conclusions will be drawn about the safety and efficiency of the mentioned composites.

**Acknowledgements:**

Partial financial support is acknowledged from the European Union, PON Research and Innovation 2014-2020 – Project: “E-Design. Combinazione di design, elettronica e materiali multifunzionali per nuovi componenti estetici” ARS01\_01158.

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**ANALYTICAL SPECTROSCOPIC INVESTIGATIONS ON NANOPARTICLE/ PHOSPHOLIPID MEMBRANE INTERACTIONS**

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Metal nanoparticles (MeNPs), such as copper nanoparticles (CuNPs), are increasingly emerging as antimicrobial agents in real-life applications and industrial goods. For this reason, the scientific community is even more involved in the study of their ecotoxicological effects. Particularly, the well-known health risks related to the NP size should be considered. MeNPs should be investigated not only in terms of their direct biological effects, but also for the actual processes dictating their human bioavailability. To this end, liposome nanoparticles have been consolidated as biomembrane artificial model to explore the potential *in-vitro* supramolecular interactions of contaminants (e.g., inorganic nanostructures) with the phospholipids of biological membranes. In particular, NP-membrane interaction can induce membrane deformation, NP absorption or wrapping, depending on their properties [1]. This contribution presents an analytical chemistry holistic approach for assessment of NP-liposome interactions. CuNPs were firstly synthesized and thoroughly characterized by means of spectroscopic (UV-Vis, FT-IR, XPS) and morphological (TEM) techniques. Then, a fluorescence spectroscopic investigation of the interaction of CuNPs with liposomes was carried out. In particular, soybean phosphatidylcholine-based liposomes tailored with two embedded fluorescence membrane probes, Prodan and Laurdan [2,3], were subjected to a given concentration of CuNPs. Such probes are able to anchor the phospholipid bilayer at different depths. Laurdan locates inside the phospholipid, while Prodan is located closer to the aqueous surface of the bilayer. Following their fluorescent properties, low-resolution changes in bilayer organization can be traced back. Generalized polarization (GP) measurements were exploited for monitoring the probe fluorescence emission over time. Alterations in lipid ordering were observed, suggesting an enhancement of lipid packing and the order at the glycerol level with concomitant membrane dehydration with the presence of CuNPs. The methodology will be used to screen the potential risk of different antimicrobial MeNP systems.

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**PHOTONICS FOR HEALTHCARE: MULTISCALE CANCER DIAGNOSIS AND THERAPY**

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The aim of the PHAST (Photonics for Healthcare: multiscale cancer diagnosis and Therapy)-ETN action is to develop an innovative training programme in the field of biophotonics. An early diagnosis and targeted treatment of cancer, which are of great importance to the European community, require the development of innovative diagnosis tools and non-invasive methods to monitor a therapy response. The main scientific goal of the PHAST-ETN action is to develop multiscale advanced photonic technologies for the diagnosis of cancer in vitro and in vivo and the monitoring of therapy for personalized medicine. Raman based technologies (e.g., resonance Raman, SERS, CARS, SRS etc.), in combination with innovative chemometric approaches and innovative fibres to bring laser light to the point of interest and to collect the Raman scattered light, have shown their great impact on biomedical research since a Raman spectrum provides a molecular fingerprint of any biological sample. A first activity regards the study and development of novel SERS-based optofluidic devices, particularly biological protocols consisting in surface functionalization of metal-dielectric nanostructures for the detection and quantification of tumour biomarkers aimed to perform early cancer diagnostics via a liquid biopsy approach. This activity merges technologies devoted to synthesis of innovative SERS nanostructures and/or labels and surface biofunctionalization as well as novel microfluidic devices. A second direction concerns the study and development of Standard Operating Procedures (SOP) for point-of-care RS to detect cancer markers in body liquids. Here the biggest challenges lie on the systematic evaluation of reproducible Raman sampling methods. This liquid biopsy approach will open new, cheaper and less intrusive ways to diagnose cancer or assess how cancer responds to treatment. The main results obtained so far regarding the analyses carried out will be reported and discussed.

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**NOVEL CMC-BASED HYDROGEL EMBEDDING BERBERINE:  
ANALYTICAL INVESTIGATION AND WOUND HEALING STUDY**

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Wound healing is a normal biological process, achieved through four subsequent stages: haemostasis, inflammation, proliferation, and remodelling [1]. Developed wound dressings have to support skin regeneration processes, avoiding infection of the healing wound. Since proper hydration of the wound bed is compulsory to achieve complete tissue regeneration, hydrogels represent attractive tools for innovative wound dressings [2]. Moreover, they could be loaded with bioactive molecules, improving the delivery through the deepest skin layers. In this work, an innovative carboxymethyl cellulose (CMC) based hydrogel film has been designed to locally release berberine [4, 5]. A detailed analytical characterization by means of XPS, FTIR/ATR, TGA and SS-NMR analyses has been carried out. A jacketed Franz cell was exploited to assess *in vitro* skin permeation of berberine eluting from the film. Quantification of berberine release was performed by HPLC. Different kinetic models have been used to describe the experimental release kinetics. Swelling studies of the hydrogel, both in free conditions and under an external pressure, have been carried out. Moreover, the *in vitro* antioxidant activity by DPPH and ABTS assays as well as enzymatic biodegradation by Lysozyme of the proposed material have been studied. Finally, its wound healing capabilities were investigated, evaluating the effect of these customized hydrogels on HaCaT and NhDF behavior both in normal and oxidative stress conditions, evidencing the potentiality of this hydrogel for chronic wound treatment.

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**REDIRECTING ARGENTOMETRIC TITRATIONS TO THE SCALABLE AND GREEN PRODUCTION OF NANOANTIMICROBIALS**

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Alarmingly, more and more infections are becoming resistant to antibiotics, which poses a great impact on the economy, health, and society. Metal-based NanoAntiMicrobials (NAMs) are broad spectrum biocidal additives that efficiently inhibit or eradicate communities of pathogenic bacteria so-called biofilms. However, nanotoxicological issues are limiting the applications of ultrafine metal nanoparticles. Alternative sources of bioactive metal ions are being sought in the areas of coordination polymers, ionic-exchangers, insoluble salts, etc. Particularly, the use of intrinsically insoluble AgCl in place of elemental Ag could increase the level of control over the extent and rate of generation of bioactive species, by providing a thermodynamically-controlled release of Ag<sup>+</sup> ions.

The goal of the present study [1] is to redirect a standard argentometric titration to develop green, reproducible, scalable, and synergistic AgCl/Benzyl-hexadecyl-dimethyl-ammonium chloride (AgCl/BAC) colloidal NAMs, killing multi-resistant pathogens. Morphology and stability of the nanocolloids were investigated as a function of different titration fractions. DLS was used to observe in real-time AgCl NPs formation surrounded by BA<sup>+</sup> species. Particle size distribution and hydrodynamic diameter predict the formation of BAC layered structures around NPs. SAXS measurements suggested the formation of AgCl-BAC NPs as core-shell structures in the aqueous medium. NAMs were further characterized by TEM, XPS and UV-Vis techniques. Our experimental evidence support the morphological stability of the nanocolloids, while higher positive zeta potential values anticipate long term antimicrobial effect. Ion release kinetics from water-insoluble AgCl/BAC/polymer coatings were obtained by ETAAS. The controlled Ag<sup>+</sup> release reached a plateau at around 0.2  $\mu$ M in about 4 hours, which is enough to have mild antimicrobial action without toxic effects towards humans. The material can find application in smart food packaging and bio-safe medical devices.

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**SPECTROSCOPIC INVESTIGATION OF INNOVATIVE SYNERGISTIC COMPOSITES FOR ACTIVE PACKAGING APPLICATION**

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Biofilms development and rise in number of resistant bacterial strains contamination cases give the motivation for creation of novel safe antimicrobial materials. Some natural non-toxic components like Chitosan (CS) are widely used in various forms, e.g. sponges, gels and films in many industries. The latest have found application as a packaging material in food industry, due to the film-forming and intrinsic antimicrobial properties, to prevent spoilage of products caused by pathogenic microorganisms. Mechanical robustness of pure CS-based films is poor and there are several potential strategies to overcome this limitation. Addition of tannic acid (TA) provides beneficial effect on mechanical properties and promotes the stability in humid conditions. TA keeps innate antimicrobial properties even upon cross-linkage with CS, the resulting film has improved physical performance and enhanced antimicrobial potency. Combatting antimicrobial resistance (AMR) is a challenging task, since microorganisms develop AMR faster than the new antimicrobials being discovered. Addition of inorganic nanophases, e.g. silver nanoparticles (AgNPs), can potentially prevent fast resistance development. It provides prolonged antimicrobial effect due to the release of silver ions and generation of reactive oxygen species (ROS). In situ generated AgNPs, which are embedded in biocompatible matrix, add prolonged synergistic effect against common food-borne pathogens. These reinforced hybrid films were developed in a fast and scalable process and characterized by various spectroscopic (ex. FT-IR, XPS) and by electron microscopy techniques in order to better understand structure-related properties. Antimicrobial potential was tested for the selected candidates.

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**SPECTROSCOPIC CHARACTERIZATION OF ANTIMICROBIAL AgCl PARTICLES SUPPORTED ON MONTMORILLONITE**

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If antimicrobial drug-resistant is not tackled now, 20 people could die every 1 minute by 2050 worldwide [1]. Bacteria have a propensity to colonize on both biotic and abiotic surfaces forming so called biofilms, and obtaining peculiar resistance mechanisms. One of the potential routes in racing against antimicrobial resistance and harmful biofilms is the continuous development of synergistic hybrid nanoantimicrobials.

Silver halides are considered as potential antimicrobial agents, which are effective in killing both airborne and waterborne bacteria by providing a tailored concentration of biocidal Ag<sup>+</sup> ions into target microorganisms [2]. However, one of the remaining challenges is how to recycle the bioactive colloids. In this study, AgCl nanoparticles (NPs) are deposited onto inorganic substrates such as montmorillonite (MMT). This paves the way to synergistic antibacterial hybrid nanocomposites, providing a slow and intrinsically limited release of Ag<sup>+</sup> species, in order to obtain a prolonged antibacterial activity and overcome toxicity towards humans. The synthesis of AgCl/BAC NPs was carried out in the presence of either MMT or Copper-exchanged MMT. AgCl NPs were entrapped onto MMT substrates based on electrostatic interactions. The use of copper exchanged MMT aims at the synergistic enhancement of the ultimate biocidal effect of the material. Analytical characterization of the nanocomposite was performed by TEM, z-potential, XPS, UV-Vis, and Infrared spectroscopies. The nanocomposite is expected to be an additive for active food packaging, aiming at long lasting bacteriostatic effects, based on the active species loading.

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**ANGLE-RESOLVED XPS: A METHOD FOR THE INVESTIGATION OF POLYMER FUNCTIONALIZATION**

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Nowadays, the food industry interest in the development of new antimicrobial active food packaging is increasing. One method to develop an antimicrobial food-packaging, might be the immobilization of antimicrobial agent onto the polymer surface [1]. In the literature, the functionalization of medical grade PVC surface with polyhexamethylenguanidine (PHMG) as an antimicrobial agent [2] was achieved by grafting (3-mercaptopropyl) trimethoxysilane (MPTMS) to PVC, followed by the reaction with aminopropyltriethoxysilane (APTES) and with glutaraldehyde as a crosslinker. There is the need of characterizing these layers and surface analysis techniques and in particular ARXPS is acknowledged to be one of the most promising for the investigation of multilayer structures.

In the present work, an analytical strategy is proposed for the characterization of the first steps of the surface functionalization of food-grade polyvinyl chloride (PVC). X-ray photoelectron spectroscopy (XPS), both in the standard mode and in angle-resolved XPS (ARXPS), were used for investigating the mechanism of surface functionalization. Food-grade PVC samples were functionalized with (3-mercaptopropyl) trimethoxysilane (MPTMS) in ethanol 96% v/v (ethanol is used here since this solvent should not damage the polymer contrary to acetone). The MPTMS molecules form covalent bonds to the PVC surface by a nucleophilic substitution reaction. Functionalization requires a uniform and complete coverage of the sample surface with the MPTMS molecule. This was checked with ARXPS measurements; the relative depth plot showed the OCH<sub>3</sub> group at the surface, silicon and sulphur located at intermediate depth. Chlorine (from the PVC substrate) was detected at low concentrations, indicating that the thickness of the MPTMS layer was lower than 5.6 nm. It can be concluded that with a treatment of 1 hour in a 10% v/v solution of MPTMS in ethanol, the first step of surface functionalization with best coverage can be obtained.

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**USE OF FOURIER TRANSFORM INFRARED (FT-IR) SPECTROSCOPY FOR THE POLYMERIC COMPOSITION ASSESSMENT OF MICROPLASTICS INGESTED BY MEDITERRANEAN FISHERY RESOURCES**

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In the last decades, there has been an increasing scientific interest on the environmental threat due to microplastics (MPs, plastic particles lower than 5 mm). Because of their chemical and physical properties, these “emerging” contaminants are ubiquitous in the marine habitat worldwide, with potential biological and ecological impacts. MPs can be ingested by marine fauna and transferred along the food-web, causing physical/mechanical damages in tissues and inducing toxicological harm. Marine Strategy Framework Directive guidelines suggest to define the trends of marine plastic ingestion in bioindicator species from European waters and to identify the MPs polymer composition in the size range 0.1-5 mm, using spectroscopy techniques. In this framework, the present study aims to assess the polymeric composition of MPs ingested by some fishery resources (*Boops boops*, *Coryphaena hippurus*, *Scyliorhinus canicula*, *Galeus melastomus*, *Raja clavata*, *Octopus vulgaris* and *Sepia officinalis*) sampled in the Gulf of Patti (southern Tyrrhenian Sea, Sicily), through one of the most used spectroscopy techniques. MPs were isolated from the gastrointestinal tracts and analyzed by ATR-Fourier transform infrared (FT-IR) spectroscopy supplied with specific polymer libraries and the level of certainty to match the sample spectrum with reference spectra was set up to > 70%. The main polymer types identified were rubber (58%), polyethylene (55%), polypropylene (40%), polyethylene terephthalate (30%) and polyvinyl chloride (17%). This analysis allowed to characterize the MPs polymeric composition in the study area, underlining that the elastomers, grouped in the rubber category, were the most

frequent MPs. This finding may be linked to motorway-related pollution through runoff processes in the coastal area near the Gulf of Patti.

## SURFACE CHEMICAL CHARACTERIZATION OF MnO<sub>2</sub>-BASED CATALYSTS

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In this study, we report on the analytical characterization of different MnO<sub>2</sub> – based catalysts and the correlation between the surface properties and their catalytic activity. High Energy Milling (HEM) was used to prepare MnO<sub>2</sub>/CeO<sub>2</sub>, MnO<sub>2</sub>/CuO, and MnO<sub>2</sub>/CuO/CeO<sub>2</sub> mixed oxides. A second route using Microwave-Assisted Hydrothermal (MWHT) was also employed to prepare MnO<sub>2</sub>@CeO<sub>2</sub>, obtaining a core-shell catalyst.

All the oxides were tested in the selective aerobic oxidation of furfural into furoic acid. X-ray photoelectron spectroscopy (XPS) analyses showed that both commercial MnO<sub>2</sub> and HEM prepared mixed oxides, present on the surface a certain abundance of Mn(III) species, which is less active in the furfural oxidation. Noteworthy, calcination of the samples in air led to an increased amount of manganese in lower oxidation states [Mn(III) and Mn(II)], highlighting the MnO<sub>2</sub> instability at high temperature [1,2]. This behaviour was well correlated with low performance in the catalytic tests.

XPS on MWHT prepared MnO<sub>2</sub>@CeO<sub>2</sub> revealed only Mn(IV) species on the surface, highlighting its excellent catalytic properties [3].

In conclusion, XPS, along with EDX and TPD analyses performed on all the catalysts, were used to explain the different behaviour of the catalytic materials, in terms of activity, recoverability, and reusability.

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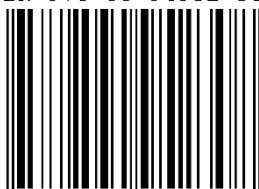
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